Summary of thesis

Energy metabolism studies with sheep

by

N.McC. Graham

As the population of the world increases and man's standard of living rises it becomes increasingly important that the world's supplies of animal feedingstuffs should be used efficiently and that waste should be minimised. If this is to be accomplished the nutritive value of feedingstuffs must be known accurately. The work described in the present thesis is part of extensive investigations that are now being made to obtain precise information on the energy metabolism of farm animals and on the ability of different feedingstuffs to satisfy their energy requirements for maintenance, for growth and for the production of meat, milk and wool. The object of the present experiments was the development of a reliable method that could be used extensively for the determination of the net energy values of animal feedingstuffs. Sheep were used as the experimental animals and closed-circuit respiration calorimetry was used to measure their energy exchange.

The thesis is divided into three main parts. In the first part a description is given of the experimental methods and of the closed-circuit respiration apparatus. The energy retention of the sheep was calculated as the difference between the energy intake and energy losses (Respiratory Quotient or R.Q. method) and also as the energy stored in body fat and protein (C/N method). The relative merits of these two methods are discussed.

The second part of the thesis describes the five experiments comprising the main part of the work. The more important results are summarised in tables in the text, and the detailed results are available for reference in a series of tables in an appendix. In the first experiment the heat productions of eight sheep were measured and it was shown that the sheep used for this type of work must be carefully trained to accustom them to the experimental routine in order to ensure that their basal metabolism remains reasonably constant. The second experiment showed that when one type of

feedingstuff is to be tested immediately after another, the sheep must be fed for at least 10 days on the new ration before measurements of energy exchange are made.

The results of the third experiment made it clear that the net energy value of a given weight of dried grass is not constant but that it decreases as the amount eaten is increased. This is due mainly to the fact that at the higher levels of feeding the digestibility of the dried grass is reduced and the amount of heat produced from the digested portion is increased. It was found that there was a curvilinear relationship between energy retention and energy intake, and an exponential equation was derived to describe that relationship.

The effects on the energy exchange of feeding dried grass in different physical forms and of altering the frequency of feeding was studied in the fourth and fifth experiments. It was found that the net energy value of dried grass was not altered by the process of grinding and cubing except at very high planes of nutrition. Frequency of feeding also had no significant effect on the utilisation of the energy of chopped dried grass although the methane production of the sheep was slightly less when they were given one large meal each day than it was when they were given the same amount of grass per day in four small meals.

In the third part of the thesis the results and methods are discussed and the net energy values which were obtained by direct determination are shown to differ markedly from those computed from digestibility data by the methods of Kellner and Armsby. The causes of the disagreement are discussed and a method of expressing net energy values to take account of variations in level of feed is described. The work has led to the conclusion that net energy values can be measured to within $\pm$ 2% by the respiratory quotient method using the apparatus and techniques described but in order to obtain this accuracy, results must be obtained for three widely-separated planes of nutrition (one of which could be the fasting level) with at least four sheep; with only one respiration chamber the determination in this way of the net energy value of one feedingstuff would require a period of 10 weeks.

ENERGY METABOLISM STUDIES WITH SHEEP

A thesis submitted to the University of Glasgow for the degree of Doctor of Philosophy in the Faculty of Science

by

NORMAN McCRACKEN GRAHAM

July, 1960.

The Hannah Dairy Research Institute,
Kirkhill, Ayr.

Acknowledgments

I wish to thank the Council and Director of the Hannah Dairy Research Institute for the facilities provided for the work described in this thesis, and Dr. K.L. Blaxter for initiating the work and for the valuable advice and encouragement that he has given at every stage. I am grateful also to several members of the staff of the Nutrition Department of the Institute who assisted from time to time with some of the routine work.

Table of Contents

Definition of terms

In discussing energy metabolism it is necessary to use several terms that are peculiar to the subject. It will probably be helpful, therefore, if these terms as used in the present thesis are defined clearly at the outset.

The gross energy of food, faeces, urine and methane is their heat of combustion.

The digestible energy of the food eaten is the gross energy of the food (total energy intake) less the gross energy of the faeces (faecal energy).

Absorbed energy is the digestible energy less the gross energy of the methane formed in the digestive tract.

Metabolizable energy is the digestible energy less the gross energy of methane and urine.

Heat production is the heat arising from the chemical and physical processes of life. It may be measured by direct calorimetry. It may also be calculated from the respiratory exchange or as the difference between energy retention (see below) and metabolizable energy.

Heat increment. When an animal ingests a quantity of food its heat production increases. The increase varies in amount according to the nature of the food and with other circumstances. The heat increment is this increase expressed as a percentage of the gross, digestible, absorbed or metabolizable

energy.

The energy retention may be calculated as the difference between the energy intake and the total energy loss (in faeces, urine, methane and as heat) or as the gross energy of the fat and protein stored in or lost from the body. Energy retention is positive when there is storage of energy and negative when there is depletion.

The net energy value (or simply the net energy) of a feed is the increase in energy retention in kilogram-calories (kcal.) per 100 kcal. of gross energy or per 100 g. dry matter ingested.

A kilogram-calorie (kcal.) is the amount of heat required to raise the temperature of 1 kg. of water from 15 to 16°C.

Summary

As the population of the world increases and man's standard of living rises it becomes increasingly important that the world's supplies of animal feedingstuffs should be used efficiently and that waste should be minimised. If this is to be accomplished the nutritive value of feedingstuffs must be known accurately. The work described in the present thesis is part of extensive investigations that are now being made to obtain precise information on the energy metabolism of farm animals and on the ability of different feedingstuffs to satisfy their energy requirements for maintenance, for growth and for the production of meat, milk and wool. The object of the present experiments was the development of a reliable method that could be used extensively for the determination of the net energy values of animal feedingstuffs. Sheep were used as the experimental animals and closed-circuit respiration calorimetry was used to measure their energy exchange.

The thesis is divided into three main parts. In the first part a description is given of the experimental methods and of the closed-circuit respiration apparatus. The energy retention of the sheep was calculated as the difference between the energy intake and energy losses (respiratory quotient

or R.Q. method) and also as the energy stored in body fat and protein (C/N method). The relative merits of these two methods are discussed.

The second part of the thesis describes the five experiments comprising the main part of the work. The more important results are summarised in tables in the text, and the detailed results are available for reference in a series of tables in an appendix. In the first experiment the heat productions of eight sheep were measured and it was shown that the sheep used for this type of work must be carefully trained to accustom them to the experimental routine in order to ensure that their basal metabolism remains reasonably constant. The second experiment showed that when one type of feedingstuff is to be tested immediately after another, the sheep must be fed for at least 10 days on the new ration before measurements of energy exchange are made.

The results of the third experiment showed that the net energy value of dried grass was not constant but that it decreased as the amount eaten was increased. This was due mainly to the fact that at the higher levels of feeding the digestibility of the dried grass was reduced and the amount of heat produced from the digested portion was increased. In other words, there was a curvilinear rather than a linear relationship between energy retention and energy intake; an exponential equation was derived to

describe that relationship.

The effects on the energy exchange of feeding dried grass in different physical form and of altering the frequency of feeding was studied in the fourth and fifth experiments. It was found that the net energy value of dried grass was not altered by the process of grinding and cubing except at very high planes of nutrition. Frequency of feeding also had no significant effect on the utilization of the energy of chopped dried grass although the methane production of the sheep was slightly less when they were given one large meal each day than it was when they were given the same amount of grass per day in four small meals.

In the third part of the thesis the results and methods are discussed and the net energy values which were obtained by direct determination are shown to differ markedly from those computed from digestibility data by the methods of Kellner and Armsby. The causes of the disagreement are discussed and a method of expressing net energy values to take account of variations in level of feed is described. The work has led to the conclusion that net energy values can be measured to within ± 2% by the respiratory quotient method using the apparatus and techniques described but in order to obtain this accuracy, results must be obtained for three widely-separated planes

of nutrition (one of which could be the fasting level) with at least four sheep; with only one respiration chamber the determination in this way of the net energy value of one feedingstuff would require a period of 10 weeks.

General Introduction

If animal productivity is to be directed more efficiently to meet man's ever growing needs, it will be necessary to have precise information on the energy metabolism of farm animals and on the ability of different feedingstuffs to satisfy their energy requirements for growth and maintenance and for the production of meat, milk and wool.

The so-called digestible energy of foods, namely that part of the energy intake which does not appear in the faeces, has been used as one measure of nutritive value (45). In herbivorous animals a portion of the gross energy of the food is lost in the form of methane which is produced by fermentation in the digestive tract, so this loss has to be deducted from the digestible energy to obtain the energy actually absorbed. Again, some of the digestible energy of the food is lost in the waste products excreted in the urine, so the metabolizable energy is the gross energy intake less the faecal, gaseous and urinary losses. This metabolizable energy is a better measure of the nutritive value of the food than the digestible energy (9). In the total digestible nutrient (T.D.N.) system of evaluating feedingstuffs as used in the United States, an approximation to the metabolizable energy is

calculated from the digestible fat, protein and carbohydrate by using certain factors (71).

Even when the metabolizable energy has been obtained it is still necessary to know the proportion of this energy that is retained by the animal under different conditions in the form of body fat and protein or that is incorporated in other useful products such as wool and milk. Now it has been shown with both ruminants and non-ruminants that all the energy in the food is accounted for by a summation of the heat production, the energy of faeces, urine and digestive gases and the energy of the protein and fat stored by the animal (6, 84). Thus when the extra heat production due to the food, or heat increment, is deducted from the metabolizable energy, the remainder, or net energy, is the actual energy obtained by the animal from the food. The evaluation of foods in terms of net energy was originated by Kellner (56) and Armsby (5) at the beginning of the 20th century and modifications of Kellner's system are still in widespread use in Europe.

The determination of the digestible energy of a ration is relatively easy since it requires only the quantitative collection and analysis of the faeces and urine produced over a period while the experimental animal is on a constant diet. To obtain an approximate value for the metabolisable energy, the methane production of herbivorous animals may be

calculated roughly from the amounts of food digested (8, 45). On the other hand, the calorimetric determination of a net energy value requires in addition the measurement of the heat production of the animal at two levels of feeding. This involves the use of complex apparatus and a very considerable amount of analytical work. An alternative to the calorimetric method of measuring net energy is to be found in the use of comparative feeding trials with a single food as standard (62, 69). With this method the amount of a food is measured which stimulates the same productive effect as a fixed amount of the standard diet. These experiments have the advantage that they can be carried out under normal practical conditions and on large numbers of animals. However, the absence of details of the composition of any changes in body weight which occur during the experiments can vitiate the results and certainly leads to a lack of precision. The method has been used successfully with pigs but with other farm animals the determination of carcass composition is less practicable.

The ease of measuring digestible energy, digestible nutrients or metabolizable energy has encouraged a great deal of experimentation with many foods and with various species of animals. The results of 25,000 digestibility trials have been listed (67). By contrast only about 300 trials have

been made to measure net energy (10, 33-39, 77, 79). In addition to experimental difficulties restricting the measurement of net energy, there has been considerable criticism of its use in food evaluation, mainly on three counts (72, 91). First, it has been said that net energy values as usually measured have little practical significance because energy retention is measured under conditions very different from those on the farm. This criticism is reasonable in so far as the "climatic" environment and the expenditure of muscular energy under experimental and practical conditions are different. Secondly, it has been said that the heat production of animals is subject to so much variation unrelated to the value of the food they eat that net energy values could not have any real meaning; this so-called biological variation in the energy expenditure is affected by changes in muscular activity and by differences in energetic efficiency between animals. Since the efficiency with which the energy of food is utilized does vary from animal to animal even within the same species, instead of ignoring this fact some attempt should be made to ascribe the extent of the variation to be expected under particular circumstances. This variability remains a serious limitation on the use of any food value for the feeding of individual animals as opposed to the computation of herd requirements. The third criticism has been that the

net energy values of individual foods are so closely dependent on the balance of the nutrients they contain that unless measurements are made on balanced rations the results are of little value (5, 33, 75). However, the evidence in the literature does not show that there is a fixed level of protein or of fibre in the diet of ruminants at which energy utilisation is most efficient: it is only when there are gross deficiencies of certain minerals and vitamins that efficiency falls (17, 57). Nevertheless, until the effect on energy metabolism of combining one food with another in a compound ration has been fully investigated, only net energy values determined with complete rations will be of much use in practice.

In view of these criticisms various alternatives to net energy have been proposed. As already mentioned, the use of digestible and of metabolisable energy, and of values computed from them and even from the chemical composition of the food, has been recommended from time to time (9, 40, 48, 74, 91). In favour of this it has been said at great length that the values can be more easily and more accurately measured than net energy, and that they are not subject to much biological variation. This is manifestly true but such food values bear little relationship to the ability of the food to promote the storage or production of useful energy by ruminants, and can give very misleading comparative

figures (16, 17, 54). The ease of their measurement and their constancy has little bearing on the matter.

Conclusions

Two outstanding problems concerning food evaluation for ruminants are

(a) the determination or prediction of the value of rations relative to one another when used for a particular purpose such as growth, maintenance or milk production, and

(b) the expression of the relative efficiencies of different productive processes.

These problems may best be solved by making reliable measurements of the net energy values of foods under standard experimental conditions.

When net energy is accepted as the most reliable estimate of food value an unsatisfactory situation is seen to exist. No calorimetric determinations of the net energy values of farm foods for ruminants have ever been made in Britain and only a few elsewhere, although the basic experimental techniques were developed half a century ago. This was due partly to a lack of simple apparatus suitable for use with farm animals and partly to disagreement about the utility of the results. Consequently, foods are usually given approximate values predicted

from the results of digestibility trials or from the gross composition of the food (15, 99). Thus 286 starch equivalents have been computed by the Ministry of Agriculture (98), mainly on the basis of 76 experiments with 37 foods and a few steers carried out by Kellner more than 50 years ago (96). The planning of cropping and of feeding programmes is made much less efficient than it should be by the unreliability of these tabulated values and by the absence of up-to-date information.

It is necessary, therefore, to promote an extensive, direct evaluation of foods on a net energy basis, and if the experimental results are to be of widespread practical value their limitations must be stated exactly.

Subject of study

The purpose of the work described in the present thesis was the development of a method which would allow the net energy values of a large number of foods to be determined accurately and conveniently. To this end the effects of some controllable factors on the net energy value of dried grass for fattening sheep were examined by means of closed-circuit respiration calorimetry.

In Part I of the thesis, Chapter 1 deals with the experimental animals and their rations, and a description is given of the general experimental techniques. Chapter 2 describes the development of

the apparatus and how it was used in the present work. The analytical scheme and methods are dealt with in Chapter 3 and the methods of computing energy retention are discussed in Chapter 4.

Part II of the thesis is devoted to the actual experiments. After investigating the establishment of metabolic equilibrium (Chapter 1), a study was made of the effects on the energy exchange of

(a) level of feeding (Chapter 2),

(b) the physical form of the feed (Chapter 3), and

(c) the frequency of feeding (Chapter 4).

Part III contains a discussion of the results in relation to the object of the work. The usefulness of the measurements of the fasting energy exchange of the sheep that were made in the course of the experiments is discussed in Chapter 1. The net energy values of the various grasses are described in Chapter 2 which also contains a discussion of the accuracy of the methods of predicting net energy originally proposed by Kellner and Armsby. In Chapter 3 the errors which arose in the measurements of net energy are described and in Chapter 4 the more important results and main conclusions are laid out. Finally a brief summary of the thesis is given.

The Appendix contains tables of results that are not required in the main text itself but which some readers may wish to refer to.

Part I

Animals, rations and methods

Chapter 1

General experimental method

Animals

Sheep were used in the present work mainly because they were considered to be more suitable experimental subjects than cattle for these initial investigations of the methods of measuring net energy. In the first place, the quantities of food eaten by sheep and the amounts of excreta produced by them are easier to sample accurately than the larger amounts produced by cattle. There are also fewer technical difficulties in measuring the respiratory exchange of small animals and the cost is less. In addition, sheep have often been used in digestibility trials and have proved themselves to be adaptable to a variety of experimental conditions. By the same token, mature, castrated male sheep were used rather than rapidly-growing or lactating animals because the fattening process is a simpler productive function to study than growth or milk production.

However, the application to fattening cattle of net energy values determined with fattening sheep can be justified. Thus only small differences have been found between cattle and sheep in regard to their ability to digest a range of foods (7, 29). In addition, Graesmann has measured with various species the net energy values (for fattening) of pure nutrients

and has found relatively small differences between the values obtained with cattle and those obtained with sheep (20).

For the present work, 2-year old, half-bred by Down crosses were obtained locally, being selected for uniformity of size and general physique. All the animals were dosed with phenothiazine to control intestinal worms and between experiments they were kept in a covered pen.

Rations

Grass was chosen as the sole constituent of the experimental diet because it is the main food of ruminants. Dried rather than fresh grass was used because it stores well and can be weighed and sampled with comparative ease. However, certain difficulties were encountered. First, the leaf of dried grass shatters very easily to a fine dust which tends to be lost in handling, and so in order to minimise differences between the composition of the analytical sample and the food actually given to the sheep, care was taken to keep this loss as small as possible during the mixing, weighing and sampling of rations. Secondly, the sheep sometimes refused to eat some of the coarser material at high levels of feeding. Fortunately, the amount involved was never large enough to upset comparisons between different levels of feeding.

These difficulties largely disappeared when

grass cubes were used. With grass cubes sampling was easier and higher levels of intake were attained than with grass in the long form.

The sheep had free access to drinking water in all experiments.

Preparation of rations

The total amount of each feed needed in any experiment was mixed on a clean floor. It was then weighed into paper bags in amounts slightly larger than required to give a quarter or a half of a day's ration for one sheep. When this had been done each bag was adjusted to the exact weight and the surplus kept as a sample. When the weighing was completed this sample was divided into four lots of about 800 g. each. The dry matter content of three of these was determined, and the fourth sub-sample was milled to pass through a screen having 10 meshes per cm. and stored in an air-tight jar until it was needed for chemical analysis. This analytical sample was thus representative of the food eaten, and the amount of dry matter given to the animals each day was not affected by subsequent changes in the moisture content of the food.

Method of experimentation

The energy retention of the sheep was measured in two independent ways; first as the difference between the energy intake and the energy lost in the faeces, urine, combustible gases and as heat, and secondly as the energy stored in body fat and protein.

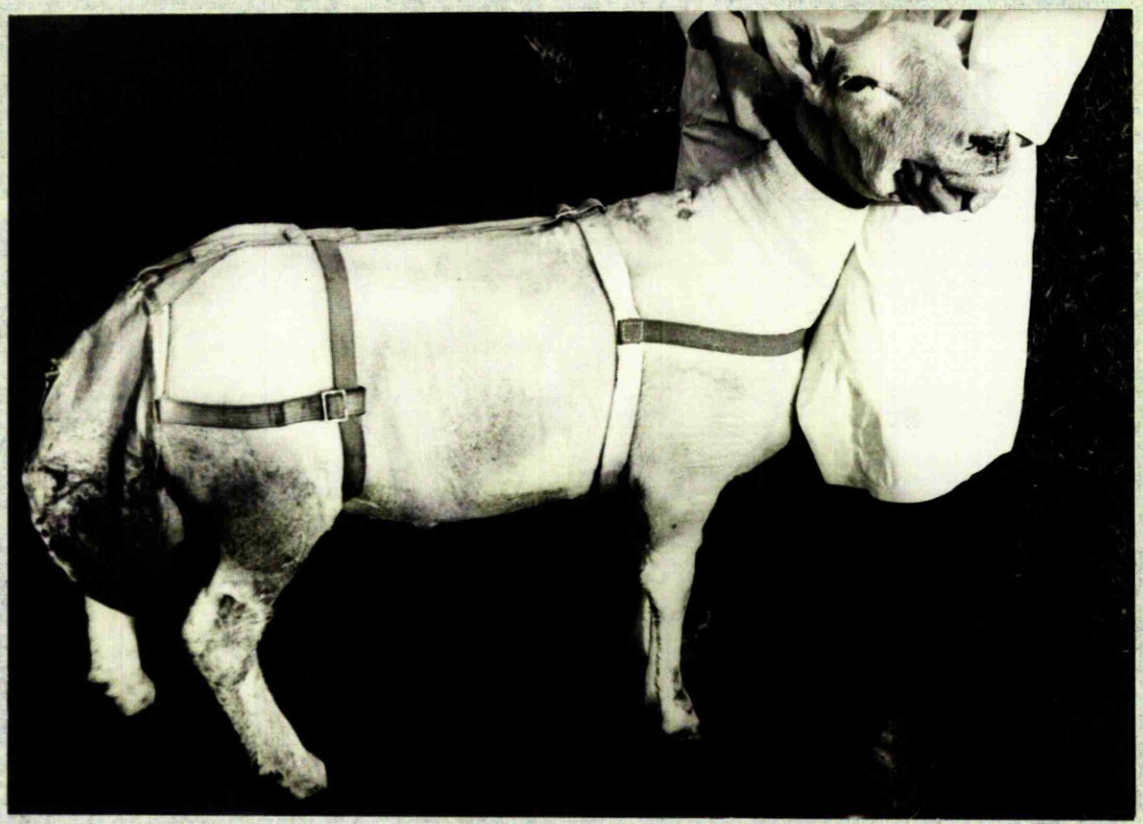


Plate 1. Wether sheep harnessed for the collection of faeces.

A bomb calorimeter was used to determine the energy content of samples of the food, faeces and urine, and two closed-circuit respiration chambers were used to measure the respiratory exchange, from which the loss of energy in combustible gas and as heat, and the loss of carbon in carbon dioxide and combustible gas, were obtained. The carbon and nitrogen retentions were determined and from these the storage of fat and protein was calculated.

The sheep were harnessed for the separate collection of faeces as shown in Plate 1.

Each experiment was divided into a number of periods corresponding to the different experimental treatments. Part of each period (the preliminary period) was allowed for the attainment of metabolic equilibrium with the ration. The sheep were then placed in the respiration chambers for at least 1 day before the 24-hr. measurements of respiratory exchange were made in the experimental period. The lengths of the preliminary and experimental periods varied from experiment to experiment. Excreta were collected, weighed and sampled each day during the experimental period and the samples were stored at 0°C until the end of the period.

Preparation of excreta for analysis

In order to reduce the total number of analyses required, each experimental period was divided into analytical sub-periods. Portions of the samples of

faeces and urine collected each day, proportional to the total amounts excreted, were mixed to give analytical samples. These analytical samples represented 1 day's excretion in Exps. (1) and (3), 2 days' excretion in Exp. (5) and 6 days' excretion in Exp. (4). The faeces were mixed in a rotary macerator, and, to the tougher faeces from sheep receiving chopped grass, it was necessary to add enough water to form a fluid suspension because such faeces were not very friable.

The specific gravity of the analytical samples of urine was measured by hydrometer to permit inter-conversion of weight and volume units.

All samples of excreta for analysis were stored at 0°C until the analyses were completed.

Chapter 2

Apparatus for measuring the energy exchange of sheep

The earliest experiments measuring the respiratory exchange of farm animals were probably those first reported in 1845 by Lassaigne (65, 64). He used a large, sealed chamber to measure the carbon dioxide production of horses, a steer and smaller animals. Three years later Regnault and Reiset described their closed-circuit respiration apparatus (68); a small animal was enclosed in a sealed box, its respiratory carbon dioxide being absorbed in alkali and oxygen being admitted to maintain pressure equilibrium.

About 1850 Pettenkofer and Voit designed an open-circuit respiration apparatus for measuring the carbon dioxide production of humans (96). The subject was confined in a chamber ventilated with outside air and his respiratory carbon dioxide was measured in a constant fraction of the air stream by absorption in alkali. Working independently at the same time, Grouven constructed similar apparatus for steers (46).

Larger apparatus for domestic animals, modelled on Pettenkofer's equipment, were soon built at four centres in Europe (81, 61, 89, 90) and in 1904 Armsby incorporated the system in his calorimeter at Pennsylvania (8). With the development of accurate

methods of gas analysis it became possible to measure oxygen consumption as well as carbon dioxide production. At intervals since this time, open- and closed-circuit respiration chambers of various sizes have been constructed in five European laboratories (19, 55, 76, 86, 100), in one Australian (67), one Japanese (62) and in three American laboratories (11, 66, 75). Two calorimeters similar to Armsby's (47, 93) and two others (25, 66) have been used in Europe.

The determination of all the respiratory components is comparatively easy with the closed-circuit type of apparatus but its use has been limited to small animals because of the difficulty of absorbing and measuring large quantities of carbon dioxide and water vapour. While there is no such constraint on the size of open-circuit chambers, the sampling of the air stream at high ventilation rates presents a difficult problem and where oxygen consumption is to be measured, extremely accurate gas analysis is required.

The main disadvantages of the direct determination of heat production by calorimetry lie in the complexity of the equipment and in the very laborious nature of the experimental work and calculations. Also, the high heat capacity of the earlier apparatus rendered its response to changes in heat

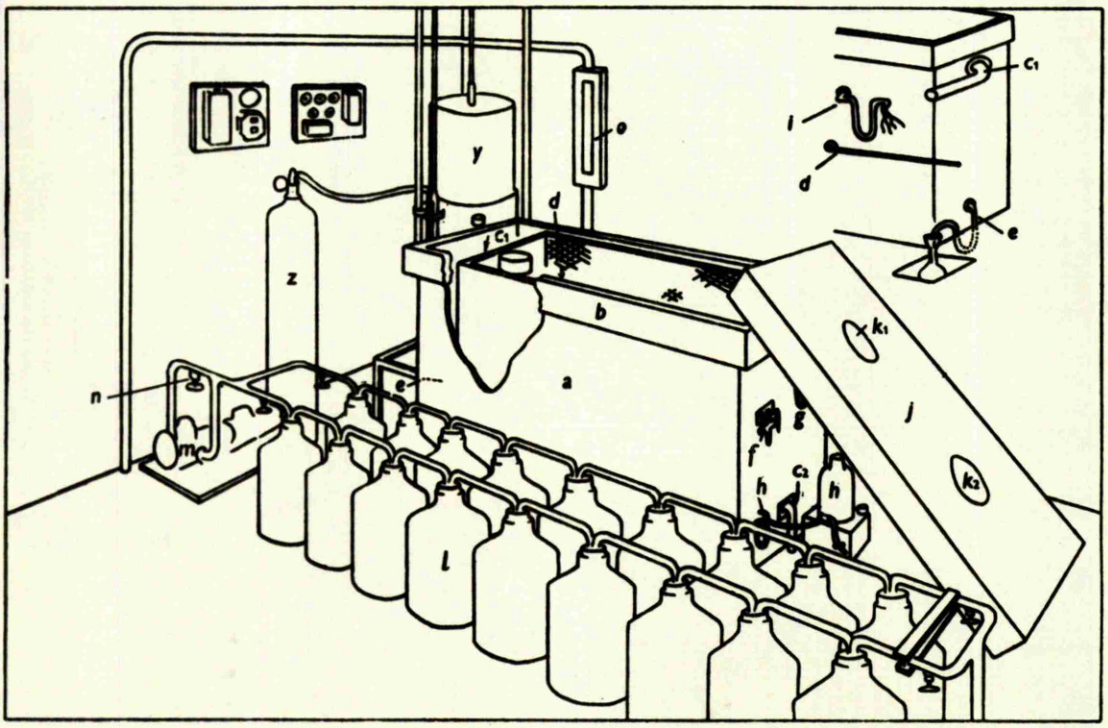


Fig. 1. Diagram of the closed-circuit respiration apparatus. (a) chamber, (b) oil seal, (c_1) and (c_2) inlet and outlet pipes for air stream, (d) oxygen inlet, (e) urine outlet, (f) air-sampling cock, (g) manometer, (h) water reservoir and inlet pipe, (i) electric leads, (j) lid, (k) inspection windows, (l) absorption train, (m) compressor, (n) by-pass valve, (o) flow gauge, (y) oxygen spirometer, (z) oxygen cylinder.

The inset is a view of the chamber from the spirometer.

production very sluggish but, this difficulty has recently been overcome (15). The satisfactory nature of "respiration calorimetry" has made the use of direct calorimetry generally unnecessary, although in some work it is still useful for checking the results obtained by the indirect method.

The apparatus used in the present work for measuring the respiratory exchange of the sheep was a somewhat modified form of the closed-circuit chamber built at the Hannah Institute for experiments with calves (10). It consisted of an air-tight chamber containing a cage for the animal. The water vapour and carbon dioxide produced by the animal were absorbed in an absorption train and sufficient oxygen was admitted from a spirometer to maintain pressure equilibrium in the system. Details of the several parts of the apparatus and of its testing, calibration and operation are given below.

The chamber

The chamber is illustrated in Fig. 1.

The chamber (a) and lid (j) were open-topped boxes, 70 x 26 x 48 inches and 72 x 28 x 6 inches respectively, made from $\frac{1}{8}$ inch sheet iron. The chamber was strengthened at the bottom edges with $1\frac{1}{2}$ inch angle iron. A trough (b), 6 inches deep x 2 inches wide, attached round the top edges of the chamber contained oil in which the sides of the lid rested, forming an effective seal. The placing of

the seal at the top, rather than at the base as in other similar apparatus, increased the rigidity of the structure and facilitated the initial testing of the apparatus for leaks since the body of the chamber could be filled with water. Inspection windows (k_1 & k_2), 6 inches diameter, were made from two layers of perspex fixed in position with nuts and bolts and sealed to either side of the lid with sealing compound. Handles were attached to each end of the lid to facilitate its removal. All joints on the structure were made by electric welding and covered with a sealing compound; the outside was then painted with red lead and the inside with white paint.

Various pipes were inserted in the side walls. These consisted of the outlet (c_1) and inlet (c_2) of the air circulating system, the oxygen inlet (d), the urine outlet (e), the air sampling cock (f), the manometer outlet (g), the drinking water inlet (h) and a pipe (i) to carry the electric leads to the heating and lighting systems of the animal cage. They were all constructed to the same general plan. The end of the pipe was threaded and a brass flange brazed on beyond the threads. A rubber washer was placed against the flange and the pipe fitted in position through a hole in the chamber. A second washer and flange were placed on the piping inside

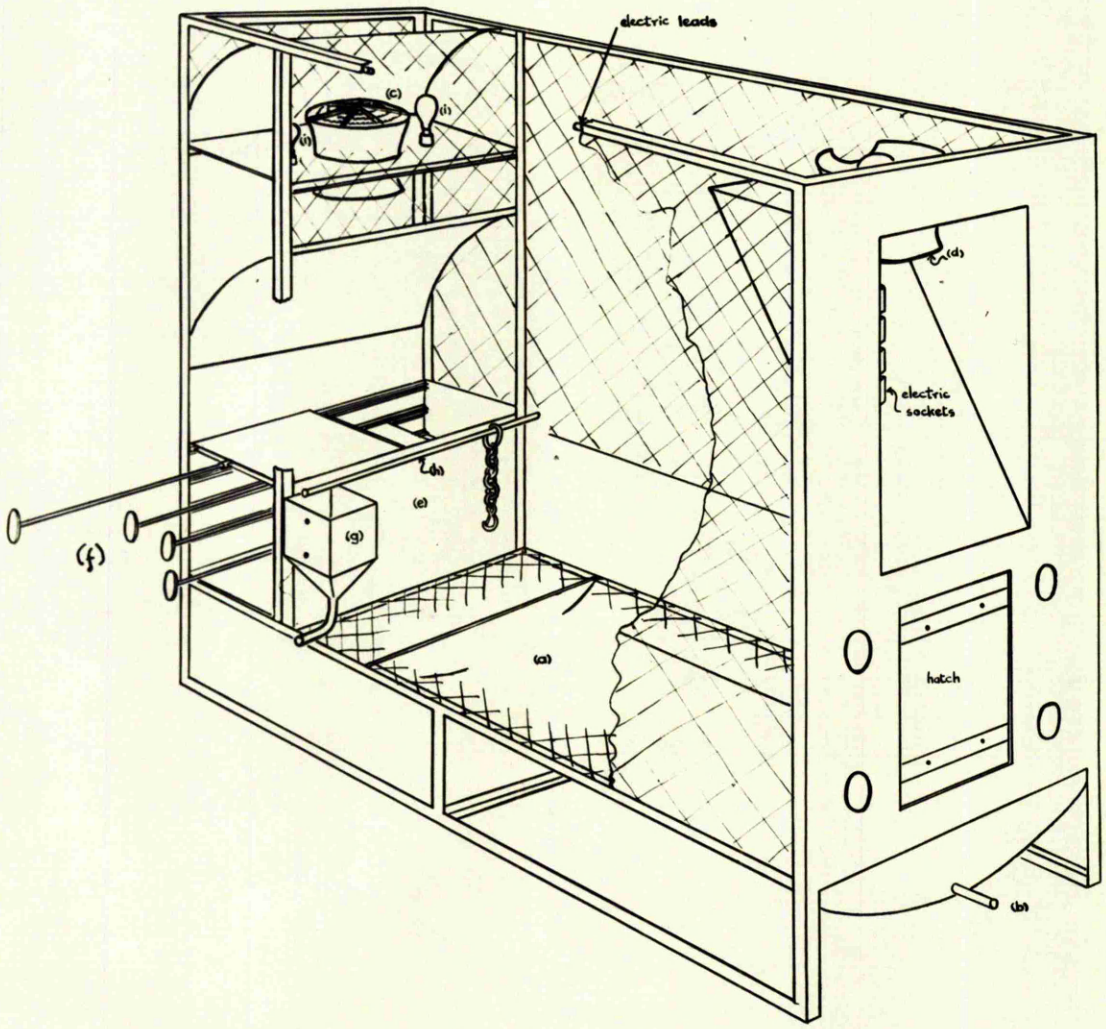


Fig. 2. The animal cage used inside the respiration chamber. (a) urine pan, (b) urine outlet, (c) fan with heating element, (d) fan, (e) food box, (f) rods to open food box, (g) water bowl, (h) rod to which the animal is tethered, (i) lighting system.

the chamber and held by a nut screwed up tightly against them. The urine outlet was U-shaped so that the liquid in it formed a seal: the multicore electric cable was sealed into a similar U-bend with paraffin wax. The urine outlet and the water inlet were approximately $\frac{1}{2}$ inch in internal diameter, the air-circulating pipes were 1 inch and the oxygen and manometer tubings were $\frac{1}{4}$ inch. The air-sampling cock was attached to $\frac{1}{8}$ inch tubes.

The animal cage

The animal cage is illustrated in Fig. 2.

The framework, which was made from $\frac{1}{2}$ inch angle iron was covered with wire grid (1 inch mesh). The floor of the cage consisted of a horizontal wire grid ($\frac{1}{8}$ inch wire, 1 inch mesh) over a urine pan made from a sheet of galvanised iron (a) which sloped to the middle and back of the cage. A $\frac{1}{2}$ inch outlet pipe (b) was soldered to the lowest point of this urine pan.

A heating element was held in slot guides immediately above the fan (c) at the head of the cage while a thermostat sensitive to temperature changes of 0.5°C was attached to the chamber at the opposite end of the cage. Two fans (c and d) gave satisfactory mixing of the air in the chamber. The ambient temperature inside the chamber could be varied from 4 to 15°C above that of the room but in the present experiments it was kept at about 30°C.

The food box (e), 10 x 10 x 10 inches, had four lids, one above the other, to provide for feeding up to four times per day. These lids moved in horizontal rails, 3½ inches apart vertically, and were opened or shut by means of ¼ inch rods (f) passing straight through grease-filled glands in the chamber walls.

The water bowl (g), 5 x 5 x 5 inches, was attached to a 5 litre reservoir outside the chamber. The top of this reservoir was just level with the top of the bowl and the water intake could be measured by filling the reservoir to a fixed mark.

The animal was tethered by a 12 inch length of chain which was attached to a ring on a horizontal rod (h) immediately behind the top edge of the feed box. The lighting system (i) consisted of a 25 watt white bulb and a 25 watt red bulb (for night observation) operated from outside the chamber.

The absorption system

Two absorption trains were provided (l, see Fig. 1) and the air flow could be directed through either of them as desired. Air circulation was by means of a rotary compressor (m) capable of delivering 500 cu. ft. of air per hour at a pressure of 5 lb. per sq. in. The rate of flow was controlled by a steam valve fitted on a by-pass (n) and was indicated on a direct-reading flow gauge (o).

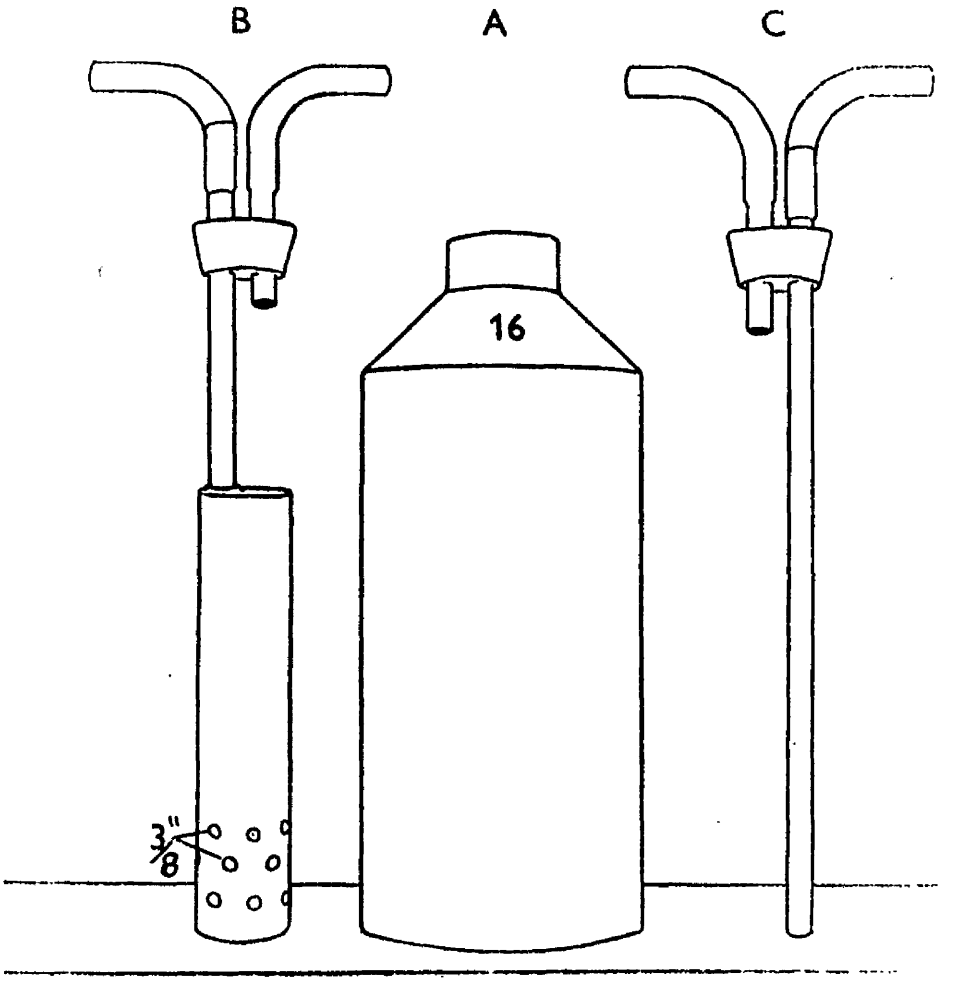


Fig. 3. Absorption bottle used with the respiration apparatus. A - polythene bottle; B - head for use with liquid potash; C - head for use with fused calcium chloride.

Each absorption train consisted of nine, 20 litre polythene bottles connected by rubber tubing. The shape of these bottles and of the two types of head for use with calcium chloride and potash, respectively, are shown in fig. 5. The first four bottles (nos. 1 to 4) were each charged with about 8 kg. of fused calcium chloride (one inch mesh) for the absorption of water vapour. Each of the next two (nos. 5 & 6) contained about 5 litres of 50% potassium hydroxide for the absorption of carbon dioxide, and the last three (nos. 7 to 9) contained calcium chloride as before in order to retain the water vapour carried over from the alkali.

The indications of satisfactory absorption of carbon dioxide and water vapour were that the carbon dioxide in the chamber did not rise above 0.6% and that bottles (4) and (9) did not gain more than 50 g. in 24 hr. It was found that the former condition was satisfied if the first potash bottle was replaced by the second, and the second bottle by a newly-charged one, each day. Bottles (1) and (7) were replaced by the succeeding ones when bottles (4) or (9), respectively, gained 50 g. or more in 24 hr. and newly-charged bottles were inserted in the vacant positions.

Methane was allowed to accumulate in the chamber and the quantity produced was estimated by analysis

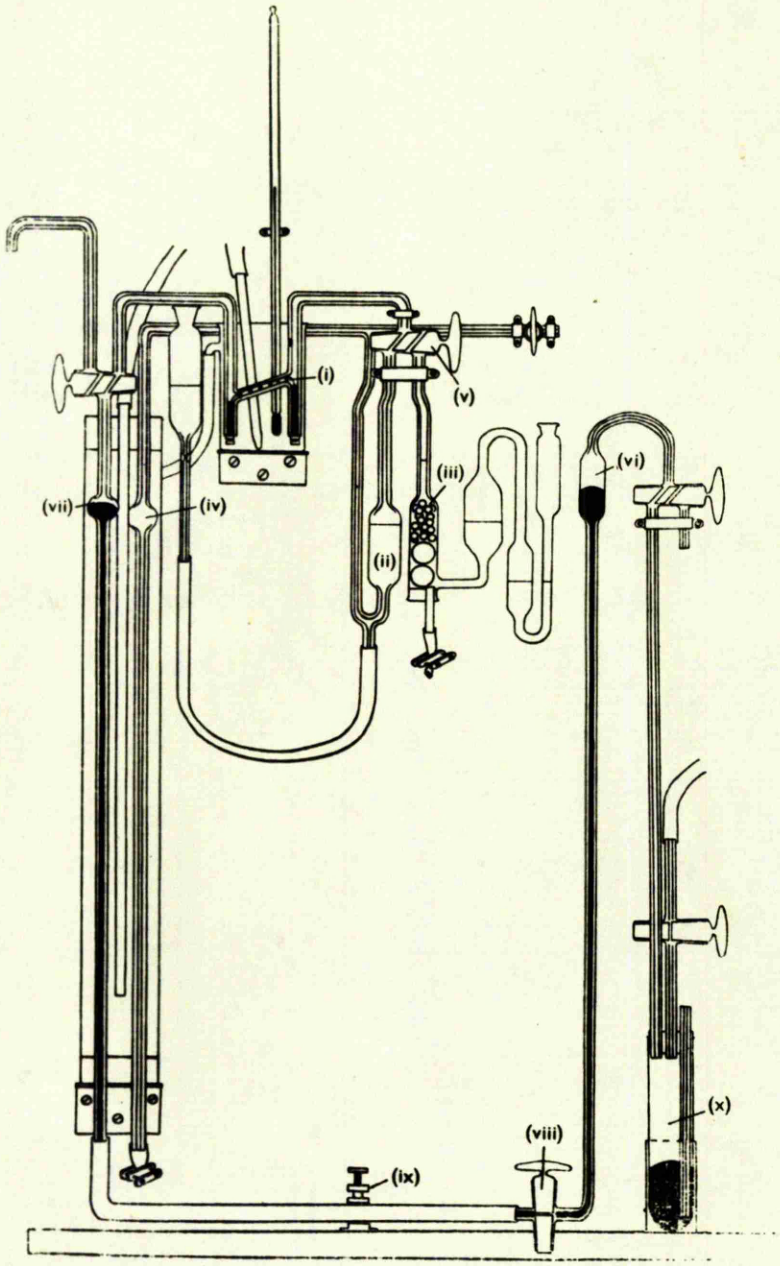


Fig. 4. Gas analysis apparatus used in conjunction with the apparatus. (i) methane burner; (ii) potash pipette for absorption of CO_2; (iii) pyrogallol pipette for absorption of O_2; (iv) compensation burette; (v) stop-cock for introduction of sample; (vi) levelling burette; (vii) measuring burette; (viii) stop-cock for coarse adjustment of mercury level in the measuring burette; (ix) screw clamp for fine adjustment of mercury level in measuring burette; (x) constant source of negative pressure to ensure that mercury in the measuring burette only falls during washing operations to the base of the bulb on the measuring burette.

of the chamber air.

Oxygen supply

Oxygen was stored at atmospheric pressure over water in a spirometer of about 160 litre capacity, described by Blaxter & Rowells (21) (see Fig. 1). The oxygen, which was always saturated with water vapour, passed into the chamber through 1 cm. of water in a bubbler which acted as a one-way valve.

Gas analysis

Any changes in barometric pressure or room temperature during the 24 hr. affected the amount of oxygen taken into the chamber; so also did the accumulation of the methane produced by the animal. Moreover, inadequate air circulation could increase the carbon dioxide content of the chamber air and so reduce the oxygen taken in. Analysis of the air in the chamber at the beginning and at the end of each 24 hr. period was therefore essential to ensure that correct estimates were obtained of carbon dioxide production, oxygen consumption and methane production.

The gas analysis apparatus built for this purpose (see Fig. 4) was basically a Haldane type. It consisted of a gas-measuring burette (vii) with carbon dioxide (ii) and oxygen (iii) absorption pipettes and the usual compensating burette (iv) to balance any small changes of temperature and pressure which occur in the course of a single analysis. The

two absorption pipettes were controlled by a single two-way cock as suggested by Carpenter, Lee & Finnerty (26).

A 15% (w/v) solution of potassium hydroxide was used to absorb carbon dioxide and a 15 (w/v) solution of pyrogallol in saturated potash to absorb oxygen. About 0.1 ml. of 1% sulphuric acid in the gas burette maintained a constant partial pressure of water vapour in the gas.

A methane burner (i) was included. It contained a spiral of platinum wire (36 S.W.G.) which could be heated to redness by means of a 6 volt battery.

The gas samples were collected over mercury in 50 ml. tonometers which also contained 3 or 5 ml. of 1% sulphuric acid.

The gas analysis apparatus made use of a special mercury levelling system. This involved an additional tube joined to the burette (vi) which could be opened alternately to the atmosphere and to a constant negative pressure (at X) supplied by a vacuum pump. Enough movement of the mercury column for efficient mixing of the gas can obtained and the risk of drawing reagents into the measuring burette was greatly reduced. The burette could be isolated from this system by shutting a stopcock (viii) and fine adjustment of the mercury level in

the burette made by means of a screw clip (ix).

Two analyses were normally done simultaneously but only one determination was carried out on each sample. Oxygen and methane could be determined with an accuracy of ±0.5% of the amounts present: the corresponding figure for carbon dioxide was ±5%.

Testing for leaks

At each stage in construction the various parts of the apparatus were tested for leaks either by filling with water (with the chamber and lid) or by inflating under water (with the absorption bottles) or by inflating, sealing and noting any loss of pressure as indicated by a mercury manometer (with the connecting pipes). While the chamber was in operation an outward leak of the circulating gases occurred occasionally, the main effect being an increase in the oxygen intake. If this increase was small, it was corrected for from the results of the gas analysis, otherwise that particular day's results had to be discarded. Leaks of this type could arise only at points in the circuit where there was a positive pressure, and were usually traced to the compressor. Sometimes a blockage occurred in one of the potash bottles due to accumulation of carbonate on the bubbler and caused an outward leak. If this leak was small it was detected by examination of the gas analysis results but a large leak immediately caused an excessive rate of oxygen intake and could

be searched for while the apparatus was running or during a brief stoppage. Large inward leaks occurred rarely because no part of the apparatus was normally subjected to a large negative pressure.

Two sources of difficulty existed. The first was the possibility of a blockage occurring in the absorption train during the night and so going undetected for several hours. If a large leak was caused and the oxygen supply exhausted, air and oil were eventually drawn into the chamber through the oil seal, spoiling the day's results and necessitating the cleaning of the chamber the following day. The second difficulty was the incidence of electric power cuts which stopped the compressor. If this happened during the night, carbon dioxide accumulated and the animal was deprived of oxygen. Fortunately both these difficulties occurred only occasionally during the present experiments.

Test for recovery of carbon dioxide

The accuracy of recovery of carbon dioxide by the apparatus was checked in the following way. Without an animal in the chamber, the air was circulated to remove all carbon dioxide, oxygen being allowed in to replace it and thus maintain the normal pressure. The absorption train was then isolated and the carbon dioxide absorbers weighed and replaced. Circulation of the air was recommenced and carbon

dioxide was admitted slowly to the circuit from a small weighed cylinder, at such a rate as to avoid a pressure rise, until about 500 g. had been introduced. When absorption of this carbon dioxide was complete, as indicated by cessation of oxygen flow, the train was reweighed. In two tests, 99% of the carbon dioxide which was introduced was recovered.

Calibration of the apparatus

The volume of the spirometer bell per cm. of its length was calculated from its dimensions and also by filling it with air displaced from an aspirator by measured amounts of water. The results of the two methods of calibration differed by less than 1%. A table of factors was then drawn up relating the volume (v) of dry oxygen, at 0°C and a pressure of 760 mm. of mercury, to the vertical movement of the spirometer bell. The equation used was:

$$V = kP \frac{273}{T} \cdot \frac{Pa - Pw}{760}, \text{ where}$$

k = the movement of the spirometer bell in cm.,

P = the mean volume of the spirometer bell per cm.,

T = the mean absolute temperature over the period of measurement,

Pw = the saturation water vapour pressure in mm. of mercury at temperature T and

Pa = the mean atmospheric pressure over the period of measurement in mm. of mercury.

The total volume of the chamber and absorption system was obtained by direct measurement, allowance being made for the volume of absorbents and other contents. It was calculated also by a gas analysis method. This consisted of introducing about 800 g. of carbon dioxide into one part of the system from a cylinder, allowing sufficient air to escape from another part of the system to maintain pressure equilibrium, and then circulating the mixed gases in the chamber through an absorption train containing no potash for a short time. A gas sample was then taken from the chamber and potash put into the absorption train. Circulation was recommenced and the carbon dioxide was absorbed and replaced by oxygen from the spirometer in order to maintain the pressure equilibrium within the system. When oxygen ceased to flow into the apparatus a second gas sample was taken. The concentration of carbon dioxide in the chamber gas was thus determined before and after a known amount of the gas was absorbed, and the volume of the apparatus was calculated from the formula

$$V = \frac{100L}{p}, \quad \text{where}$$

V = the volume of the apparatus in

Table 1. Calculation of the volume of the apparatus employed

Test	Vol. of CO_2 removed by absorption bulb or vol. of O_2 introduced from eudiometer (litres) (a)	Change in the percentage of CO_2 or O_2 in the gas (b)	Volume of gas in the apparatus (litres) $\left(\frac{100a}{b}\right)$
1. CO_2	153.8	−9.77	1519
1. O_2	93.1	+6.60	1400+
2. CO_2	197.8	−9.85	1567
2. O_2	111.6	+7.75	1440+
Volume obtained by linear assessment of the apparatus			1547
Mean, excluding the two values marked +			1554

+ See text opposite

litres at N.T.P.,

P = the decrease in the percentage of carbon dioxide in the apparatus due to its absorption and

L = the volume in litres (at N.T.P.) of carbon dioxide removed by the absorption train.

The volume of the apparatus was determined similarly from the increase in the percentage of oxygen in the gas and the volume of oxygen introduced. Table 1 gives typical results. In the examples given the values obtained from the oxygen figures were low. This is known to have been caused by the inertia of the spirometer bell which resulted in the amount of oxygen introduced being slightly too low. The volumes obtained from the oxygen values were therefore not used in obtaining the mean figure of 1534 litres shown in the table. Normally 80 litres was deducted from this mean volume to allow for the volume occupied by the sheep.

Operation of the respiration chamber

The operation of the respiration chamber can be explained by describing the work involved on the middle day of an experimental period.

At 8.30 a.m. a gas sample was taken from the chamber, the compressor was stopped and the spirometer and absorption train were isolated. A record was

kept of (a) the time of stopping, (b) the spirometer reading and temperature and (c) the atmospheric pressure.

The lid of the chamber was removed and the sheep was lifted out by means of block and tackle. The sheep was then weighed and the faeces were removed from the faeces bag into a weighed bucket. While the animal was in the weighing cage the spirometer was refilled from a cylinder of compressed oxygen, the day's food was put into the mangers and the water reservoir was made up to the mark. When the urine pan had been washed with 250 ml. of distilled water, the sheep was lifted into the chamber and the lid was replaced.

A gas sample was taken and the system was sealed when the working temperature had been reached. Before the compressor was started a note was made of (a) the time of sampling, (b) the spirometer reading and temperature and (c) the atmospheric pressure. The circulation rate was adjusted to 4 cu. ft. per min. Usually only about 10 min. elapsed between stopping and restarting the compressor.

The urine and faeces were then weighed and sampled. The urine bottle was washed out, drained and 10 ml. of preservative solution added to it in readiness for the next day. The preservative consisted of a 10% solution of a 7:1 mixture of potassium dichromate and mercuric chloride. The

absorption bottles were weighed and put in position ready for the following day.

The spirometer was refilled three times in the next 24 hr. The sheep were fed either 1, 2, 3 or 4 times per day by opening the necessary number of manger sections. The drinking water reservoir was refilled at feeding times from a 2 litre measuring cylinder. The electric light that lit the chamber during the day was switched off at 5.30 p.m. and on again at 5.30 a.m.

Two respiration chambers were used in most experiments.

Table 2. Scheme of analysis

Purpose	Analyses necessary
Direct determination of net energy values	Calorific value of the food eaten and of the faeces, urine and methane excreted. The heat production is calculated from the respiratory exchange.
	Carbon content of the food, faeces, urine and expired gases. Nitrogen content of the food, faeces and urine.
Prediction of net energy values	Organic matter, ether extract, crude fibre, crude protein and nitrogen-free-extract of the food and faeces.
Further description of the food for future reference	Cellulose and non-cellulosic fibrous in the food and faeces. Soluble carbohydrates and lignin in the food.

Chapter 4

Analytical methods

The various analyses made are shown in Table 2. Some of them were necessary for the direct determination of net energy, some for its prediction and some for obtaining a detailed description of the food. In the present chapter the accuracy of the analyses is discussed and then the methods are described in detail.

Analytical accuracy

To avoid systematic error standard compounds were analysed periodically for carbon, nitrogen and energy content. Details of this are given below. The carbon and nitrogen content and the calorific value of food and faeces, and the urinary nitrogen were determined in triplicate samples. Urinary carbon and calorific value were determined only in duplicate samples because with the relatively small amounts of carbon and energy in urine the standard of accuracy does not require to be quite so high. In these analyses the error permitted did not exceed 1% of the quantity measured except with urinary carbon and urinary calorific value where the maximum error was 6%.

Duplicates differed by less than 1% of the mean in the determination of dry matter and crude protein in food and faeces, and by less than 3% in the determination of the other chemical constituents.

Carbon

Carbon was determined by a dry combustion method (93). Samples of such a size as to yield about 0.4 g. of carbon dioxide were used. The combustion tube contained copper oxide at 700°C to ensure complete oxidation and a sintered mixture of copper oxide and lead chromate at 500°C to absorb acid oxides other than carbon dioxide. The carbon dioxide was retained by flaked sodium hydroxide (diluted with 8-16 mesh pumice stone) after water vapour had been removed by fused calcium chloride.

It was necessary to dry the urine over fused calcium chloride *in vacuo* before combustion because otherwise large quantities of water vapour blocked the combustion tube and prevented satisfactory recovery of carbon. A wet combustion method (95) failed to give reliable results due to incomplete combustion of some of the urinary constituents, especially urea. Investigation showed that no loss of nitrogen occurred during the drying process but it could not be proved that there was no loss of carbon. This was not a serious matter, however, because urine contains very little carbon relative to the amount in faeces.

At monthly intervals carbon was determined in a sample of casein as a check on the method. The standards used for testing the apparatus were oxalic acid and p-dimethyl amino benzaldehyde.

Nitrogen

The macro-Kjeldahl method was used for the estimation of nitrogen in samples sufficient to yield 20-30 mg. of nitrogen.

The digestion catalyst contained potassium sulphate, copper sulphate pentahydrate and selenium powder in a 66:10:1 mixture. Boric acid (4%) was used to neutralise the ammonia which was subsequently titrated with exactly N/10 sulphuric acid (1 ml. = 1 mg. nitrogen) in the presence of an indicator containing 0.1% of a 1:1 mixture of methyl red and methylene blue.

Accuracy was checked once per week by analysing a sample of casein and p-dimethyl amino benzaldehyde was used as a standard to test the method.

Calorific value

Heats of combustion of food and faeces were measured with samples of a size to yield about 5 kcal., using a bomb calorimeter: 10 ml. samples of urine were used. Faeces and urine were first dried over fused calcium chloride *in vacuo*.

Sufficient temperature readings were taken at 1 min. intervals before and after ignition to enable a cooling (or heating) correction to be made, using the following equation:

Corrected rise in temperature =

$$(T_{n-1} - T_0) + nV + \frac{V' - V}{T' - T}\left(\sum_{n=1}^{0} T + \frac{T_0 - T_n}{2} - nT\right),$$

where T is the temperature at the time indicated by the subscript: zero time is the point at which ignition occurs, and n is the number of minutes later at which the temperature change becomes uniform; V and V' are the average temperature decreases per minute in the period before zero time and after the nth minute, while T and T' are mean temperatures for the same periods.

With urines, because the rise in temperature of the bomb was small, a simpler equation was used viz:

$$\text{Corrected rise in temperature} = T_{n-1} - T_0 + nV,$$

the symbols having the same meaning as before.

The measurement of the sulphuric and nitric acids formed in the combustion in order to enable deduction of their heats of formation and solution from the total, was omitted because considerably less than 1% of the total heat of combustion was involved.

After combustion in the bomb the loss of weight of the sample crucible on ignition at 550°C for 2 hr. was measured. This loss was assumed to be carbon with a calorific value of 8.1 kcal. per g., and the calorific value of the sample was increased accordingly. If this correction was more than 1% of the total heat evolved the result was discarded and the determination repeated.

A high degree of accuracy was never obtained

with urine for several reasons. First, the large amount of salt present fused into a solid layer on the bottom of the crucible preventing combustion of part of the sample. Secondly, the samples were never completely dry before ignition and a layer of urine always adhered to the side of the crucible. Both of these factors led to some of the material not being burned at all. Further, the yield of calories from the largest sample that could be used was quite small and this increased the relative error. The use of cellulose pads of known calorific value to absorb the urine and facilitate drying did not give appreciably better results. However, the urinary calories constituted only a small fraction of the energy retention and so a relatively large error in their measurement could be tolerated.

Proximate principles of the food and faeces

Dry matter, ash, ether extract, crude fibre, crude protein and nitrogen-free extract were determined by the usual methods (2) with only minor modifications. Coarse porosity, sintered-glass filter sticks were used for all filtrations, with the addition of celite filter aid which had been previously ignited. These analyses provided digestibility data from which a figure for the net energy value could be predicted by methods in common use.

Soluble sugars, cellulose, furfural and lignin

These remaining analyses provided a more detailed

description of the food for future reference. In Exps. (3) and (4) cellulose, cellulosic furfural and total furfural were determined in the food and faeces so that any changes in the digestibility of the structural components of the food could be examined. Soluble carbohydrates were determined by the method of Kaile & Boyd (35), cellulose by the method of Norman & Jenkins (60), furfural by the method of Kullgren & Tyden (38) and lignin by the method of Ellis, Matrone & Maynard (31).

Chapter 4

Methods of calculating the energy retention

The energy retention of the sheep was calculated in two virtually independent ways which are now considered in some detail.

Respiratory quotient (R.Q.) method of estimating energy retention

The energy retention was calculated as the difference between the energy in the food and the energy lost in the faeces, urine and combustible gases, and as heat. The energy of the food, faeces and urine was measured by bomb calorimetry as already described. The combustible gas, determined by analysis of samples of air from the respiration chamber, was assumed to be methane with a calorific value of 9.44 kcal. per litre at N.T.P. It has been shown that methane is the only combustible gas normally produced in significant quantities in the rumen although some hydrogen is formed when realimentation succeeds prolonged starvation (61).

The calculation of heat production requires further explanation.

When carbohydrate, fat and protein are catabolised, oxygen is absorbed, carbon dioxide is exhaled from the lungs, nitrogenous compounds are excreted in the urine and heat is produced.

From the analysis of muscle Loewy (68) calculated that 1 g. of nitrogen in the urine arises from an

incomplete oxidation of protein involving the absorption of 5.94 litres of oxygen, and the production of 4.70 litres of carbon dioxide and 26.5 kcal. of heat. Thus if the loss of nitrogen in the urine is known, the total respiratory exchange and heat production may be divided into two components, representing the catabolism of protein and non-protein substrates, by means of these factors.

The complete oxidation of glucose to carbon dioxide and water gives rise to 5.05 kcal. of heat per litre (at N.T.P.) of oxygen absorbed; for fat, the average value is 4.69 kcal. With the oxidation of glucose and fat the ratios of carbon dioxide produced to oxygen used (the respiratory quotients or R.Q's) are 1.000 and 0.707, respectively. Thus the heat production due to the oxidation of a mixture of carbohydrate and fat may be calculated from the non-protein oxygen consumption and the calorific value of oxygen corresponding to the particular non-protein R.Q. (101).

This method of computation has been criticised on the grounds that a non-protein R.Q. between 0.7 and 1.0 does not mean that only fat and carbohydrate dissimilation are taking place in the body (80). Thus certain organs partially oxidise intermediary metabolites, producing R.Q's outside this range. Regarding the animal as a whole, however, by far the main source of energy is normally the carbohydrate,

fat and protein of the food or tissues and the end products of their oxidation are carbon dioxide, water and urinary constituents (89). Therefore, according to Hess' Law of constant heat summation, which states that the heat exchange in a reaction is constant, irrespective of the number of steps involved, the R.Q. method of calculating the heat production of an animal is a valid one.

There are two major difficulties in the use of this method of computing heat production. First, there is no means of distinguishing the heat arising from fermentation in the rumen. The usual way of allowing for the incomplete oxidation represented by the methane produced in the rumen is to adjust the actual carbon dioxide and oxygen exchange by the amounts of these gases that would be involved if the methane were completely oxidised. The heat production due to protein and non-protein oxidation is then calculated as already described, and the actual calorific value of the methane (9.44 kcal. per litre at N.T.P.) deducted from it (1). This final figure is arrived at by thermodynamically sound means (see Hess' Law quoted above) and is an estimate of the heat production of both the animal and its symbiotic micro-organisms.

The second difficulty arises when synthesis of fat from carbohydrate predominates, as indicated by an R.Q. greater than unity. Only very unsatisfactory

empirical methods of allowing for this are available (59), and since no very high R.Q.'s were encountered in the present work, the calculation of the heat arising from the synthesis of fat was omitted. When the R.Q. was slightly greater than 1.0 the calorific value of oxygen was taken to be 5.047 kcal. per litre (i.e. representing oxidation of carbohydrate only).

Carbon and nitrogen retention (C/N) method of estimating energy retention

The second method of estimating energy retention consisted of first calculating the protein and fat loss or storage from the nitrogen and carbon exchange using average figures for the nitrogen and carbon in body protein, and for the carbon in body fat. The energy equivalent to this protein and fat was then obtained by the use of average factors (23). This calculation is valid only if the body reserves of carbohydrate remain unchanged. Since carbohydrate storage is seldom more than 2% of the body weight (23), it is safe to assume that this condition was satisfied in the present experiments.

The ultimate justification of these methods lies in the close agreement that has been found to exist between results obtained by them and by direct calorimetry. Thus many workers have confirmed the validity of the C/N method, notably Rubner (64) with

Table 5. Examples of the calculation of energy retention by the R.Q. and G/N methods

(a) R.Q. method

Oxygen consumed	449	litres at N.T.P.	
Carbon dioxide produced ...	449	litres at N.T.P.	
Methane produced	88.5	litres at N.T.P. x 9.44*	= 836 kcal.
Urinary nitrogen	10.8	g. x 38.6*	= 439 kcal.

Oxygen corrected for protein oxidation and methane production ... 449 - (10.8 x 5.06) + (88.5 x 2)# = 415 litres

Carbon dioxide corrected for protein oxidation and methane production ... 449 - (10.8 x 4.70) + (88.5)# = 405 litres

** Non-protein R.Q. = 405 ÷ 415 = 0.980

** Calorific value of oxygen = (5.016 + 1.888 x 0.980)** = 5.020 kcal. per litre at N.T.P.

** Total heat production = (415 x 5.020) + 439 = 2519 kcal.

Energy in food	4870 kcal.
" " faeces	1245
" " urine	978
" " methane	836
Heat production	2519
Total energy loss	5578 5578
Energy retention	+ 292 kcal.

(b) G/N method

Nitrogen retention	+ 8.87 g. x 5.55##	= +85 kcal.
Protein retention ... 8.87 x 6.25##	= + 10.1 g.	
Carbon retention	+ 60.96 g.	
Carbon in protein ...10.1 x 0.525##	= + 5.25 g.	
Carbon in fat	+ 55.00 g.	
Fat retention .....55.00 x 1.86##	= + 29.5 g. x 9.37##	= +276 kcal.
Energy retention		+361 kcal.

* Generally accepted calorific values (see text).

\# The complete oxidation of one volume of methane requires 2 volumes of oxygen and gives rise to 1 volume of carbon dioxide.

** Linear extrapolation between the separate values for carbohydrate and fat.

\#\# These are average factors obtained from the analysis of body protein and fat (28).

Table 3.

dogs and Armsby (4) with steers, and further, no systematic difference has been found between the C/N and R.Q. methods by other workers or in the present experiments (see p. 102).

An example of both methods of calculating the energy retention is given in Table 3.

Part II

Experimental

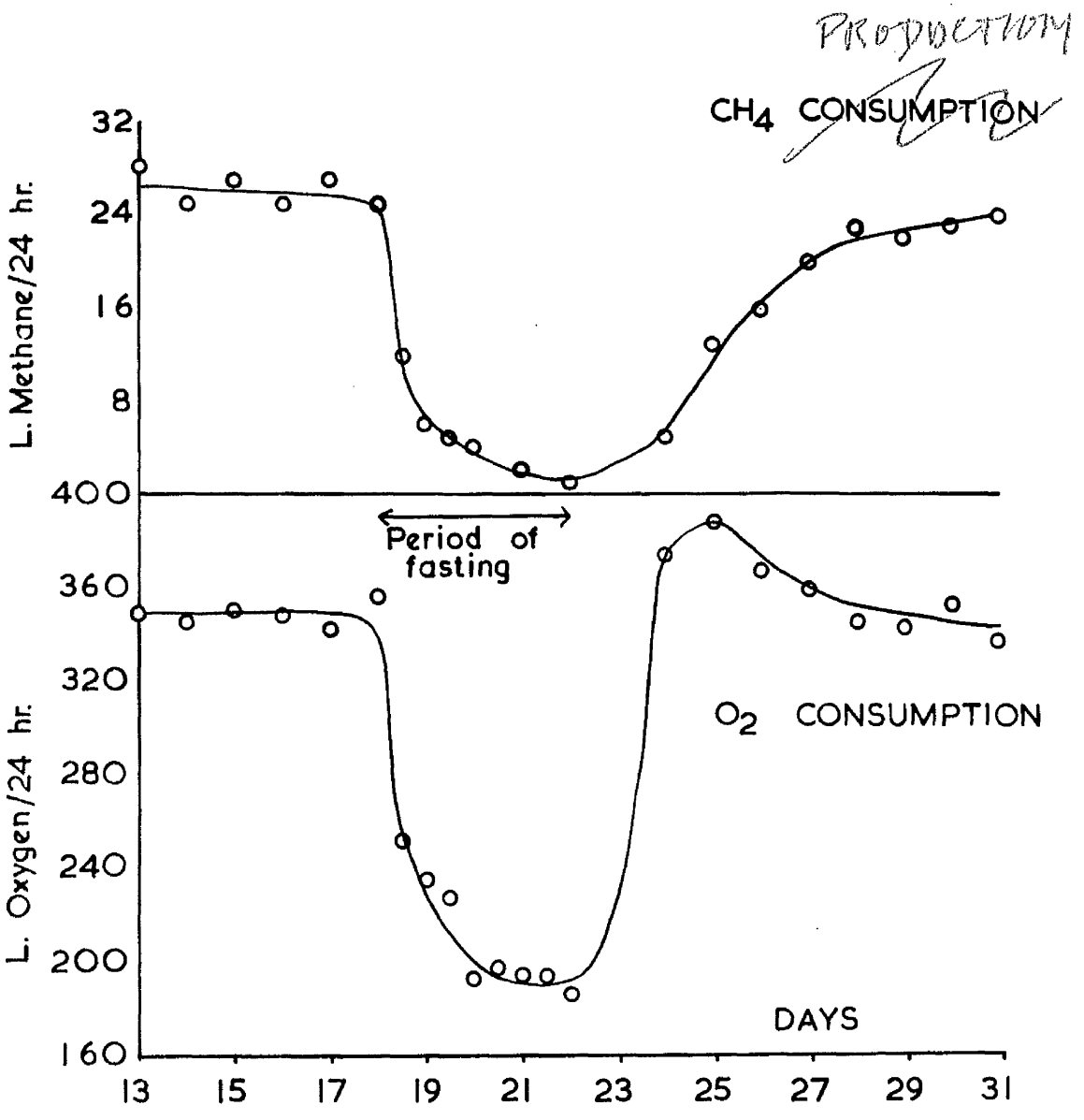
Chapter 1

The establishment of a stable energy exchange before measuring energy retention

Before the energy retention of a sheep receiving a particular ration can be measured, the energy exchange of the sheep must be stable and effects due to previous rations must have disappeared. Two groups of factors affect the stability of the energy exchange of an animal. The first is concerned with the animal's reaction to its physical environment. The second is concerned with the attainment of energy equilibrium after a change of diet. Information was obtained about both these effects in the following two experiments (Expts. (1a) and (1b)).

Experimental and results

In Exp. (1a) a study was made of the effect of the apparatus and experimental routine on the stability of the energy exchange of sheep. The heat productions of six sheep which had not previously been used for experiments were measured on the third and fourth days of fasting. After an interval during which each sheep was kept for two 6-day periods in the respiration chamber with the lid down and the air compressor running, the fasting heat productions were again measured. The measurements were repeated a third time after a second period of "training". For comparison, two sheep which had just been used in a long calorimetric experiment, and were therefore



OXYGEN CONSUMPTION AND METHANE PRODUCTION BEFORE AND AFTER A PERIOD OF FASTING

Fig. 5.

Table 4. The fasting heat production (kcal. per 24 hr.) of trained and untrained sheep

Length of present period of training (weeks)	Fasting heat production of sheep untrained initially						
							Mean
0	1290	1371	1262	1150	1089	1072	1224
4	1108	1157	1059	1018	958	1063	1061
9	1029	1528	959	970	1102	958	1089
Standard error							± 65

Length of present period of training (weeks)	Fasting heat production of sheep trained initially		
			Mean
0	1163	1085	1124
4	1208	1021	1166
9	1180	1062	1155
Standard error			± 60

accustomed to the routine, were given identical treatment and the same measurements made.

The results of this experiment, which are given in Table 6, were analysed statistically. This showed that whereas the mean fasting heat production of the six sheep which were untrained initially fell from 1264 to 1051 kcal. per 24 hr. (standard error of means ±85 kcal. per 24 hr.) as a result of the first period of training, that of the two sheep which were fully trained initially only changed from 1164 to 1103 kcal. per 24 hr. (standard error of means ±60 kcal. per 24 hr.). At the end of the second period of training the mean fasting heat productions for the two groups were 1089 ± 80 and 1155 ± 60 kcal. per 24 hr., respectively. Thus the first period of training had a significant depressant effect ($P < 0.001$) only on the fasting heat production of the sheep which were untrained initially but the second period of training had no such effect.

In Exp. (1b) a sheep which was fully trained was given 1800 g. of chopped dried grass (A) per day, and on the 11th day 24 hr. measurements of the respiratory exchange were started. Food was withheld from the 19th until the 22nd day and given again for the succeeding 12 days, measurements being omitted on the first 4 days of realimentation. The results are given in Fig. 6 where methane production and oxygen consumption are plotted against

the number of days after the recommencement of feeding. The graph shows that there was not an immediate resumption of methane production when food was given. It rose rapidly after the first day of refeeding and by the ninth day it had reached a relatively stable value which was 6% below the mean pre-starvation level. Oxygen consumption and carbon dioxide production were steady 8 to 9 days after refeeding, only differing then from the mean pre-starvation levels by about 1%.

Discussion

The results of Exp. (1a) show that it is important to train the sheep before using them for the measurement of net energy values. A decrease of 20% in basal metabolism in the first few weeks of an experiment would cause the net energy value of the food to be considerably in error. For example, the fasting energy loss might be measured first in an experiment and be 1500 kcal. per day. If 1000 g. of dried grass were then given per day, and after 2 weeks the energy retention was measured and found to be zero, the apparent net energy value of the grass would be $\left[0 - (-1500)\right]$ 100/1000 or 150 kcal. per 100 g. of grass. However, if the basal metabolism was actually 300 kcal. per day lower during the measurement of the energy retention promoted by the grass than during the measurement of the fasting energy loss, the true net energy of the

grass would be only $\left[0 - (-1800)\right]$ 100/1000 or 180 kcal. per 100 g.

The results of Exp. (1b) show that the fermentation in the rumen and the animal's respiratory exchange were normal 9 or 10 days after the end of starvation. Since the sheep recovered from this drastic change (i.e. from starvation to a high level of feeding) in 9 or 10 days, it might be expected that metabolic measurements made 10 days after a smaller quantitative or qualitative change of ration, would give the true effects of the food and not be influenced significantly by the reaction to the change itself. This conclusion is further justified by the results of studies by Blaxter, Graham & Wainman (20) of the rate of passage of food through the digestive tract. These studies showed that 99% of the residue of a meal of chopped dried grass was eliminated in 10 days and that the residue from a meal of dried grass cubes was eliminated in 8 days.

Conclusions

It was concluded that a long period of training of the experimental animals is necessary to eliminate the effects of nervousness on their basal energy expenditure. For 7 or 8 weeks before any experiment to measure the net energy value of a food the sheep should be harnessed and kept in individual cages. They should also be handled frequently and given

several periods of isolation, each lasting 2 or 3 days, in the respiration chambers. Starvation for 4 days in the chamber is also helpful in the training process.

It was further concluded that 10 days after changing the amount or quality of dried grass given to a sheep, the metabolic effects of the previous ration and of the change itself are negligible. Therefore before measuring the energy retention promoted by a particular ration, the sheep should be given this ration for at least 10 days.

Chapter 8

The effect of level of feeding on energy utilization (Exp. (2))

Earlier workers carried out experiments to study the effect of food intake on energy utilization of man (46), cattle (34, 38, 39 & 75), rats (40), rabbits (50, 97) and sheep (70). The results were generally expressed in terms of the relationship between heat production and metabolizable energy. They showed that when food intake was below that required to maintain energy equilibrium, the increment of heat per kcal. of metabolizable energy increased with level of feeding. However, when food intake was sufficient to allow energy storage, the curvilinear trend was noticeable only at levels of feeding near the maximum appetite of the animals (34). Marston (70) interpreted his own results with sheep and those obtained by other workers with cattle as showing complete linearity of the relationship between heat production and metabolizable energy above energy equilibrium. The equation he used to describe the relationship may be written as,

$$H = B + M(1 - K_A)$$

where H is the heat production and M is the metabolizable energy in kcal. per kg.$^{0.75}$ of body weight per 24 hr., and where K_A is a constant depending on the food and B is a constant regarded as representing

the heat production at the fasting level less the heat increment due to the catabolism of body substance. However, high food intakes were not achieved in Marston's experiments and recalculation of the results by Kellberg (90) did, in fact, show slight curvilinearity.

If Marston's linear equation were valid it would have an important bearing on the determination of net energy values. Thus the net energy value of a food (NEV) may be written as,

$$NEV = \frac{dS}{dG}$$

where dS/dG is the increase of energy retention per unit increase of gross energy intake. This may be expanded to give,

$$NEV = \frac{dM}{dG} \cdot \frac{dS}{dM}$$

where M refers to the metabolizable energy, and since S = M - H where H is the heat production, then,

$$NEV = \frac{dM}{dG} \cdot \frac{d(M - H)}{dM} = \frac{dM}{dG}\left(1 - \frac{dH}{dM}\right).$$

Now from Marston's equation

$$\frac{dH}{dM} = 1 - K_A \quad \text{at levels of feeding above maintenance,}$$

therefore,

$$NEV = \frac{dM}{dG} \cdot K_A \text{ for levels of feeding above maintenance.}$$

Thus to obtain the net energy value of a food for levels of feeding above maintenance it would be necessary to measure dM/dG and K_A. Now dM/dG is the increase of metabolizable energy per kcal. of gross energy intake and its measurement does not require calorimetry. However, K_A is the energy retention at any one level of feeding less the constant B, all divided by the metabolizable energy at this level of feeding: the measurement of K_A thus involves calorimetry, but only at one level of feeding. Even though the level of feeding at which energy retention was measured was not a high one, it could be well above the fasting level corresponding to B and hence K_A could be calculated accurately. By contrast, the classical method of determining the net energy value of a food involves the measurement of energy retention at two levels of feeding, usually maintenance and one higher level. The difference between these energy retentions divided by the difference in gross energy intake is the net energy value. In considering this method it is important to note that the two levels of feeding are bound to be relatively close together since they are limited by the maintenance level in one direction and by the maximum appetite of the animal in the other. The result is that

the estimate of net energy obtained by this method is correspondingly less accurate than that obtained by the method which is based on the determination of K_A and M/C.

The purpose of the present experiment (Exp. (2)), a description of which has already been published (16), was to clarify the relationship between energy utilisation and level of feeding in order to determine whether net energy could be measured in the new way described above.

Experimental

Two sheep (nos. 3 & 5) and two grasses were used. Chopped dried grass (B) was given to sheep 3 and cubed dried grass (C) to sheep 5. The chemical composition of these grasses is given in Appendix Table 1.

The grasses were given in consecutive periods at levels ranging from 500 g. per day to maximum appetite in steps of 350 g. per day. At the end of the experiment both sheep were given 500 g. per day and were then fasted, their fasting energy loss being measured on the 3rd and 4th days of fasting. Each period lasted 14 days; long enough to enable the animals to become adjusted to the level of feeding but sufficiently short to avoid major changes in body weight. During the last 5 days of each period the sheep were placed in the respiration chamber. On the first of these 5 days the apparatus was run

Table 8. Constants in the linear equation* relating heat production (H) to metabolizable energy (M) at positive energy balances

	E_A	B (kcal/kg$^{0.75}$/24 hr.)	No. of determinations used to obtain the constants
Group B (clipped) Sheep B	0.50	80.4 ± 6.9	5
Group D (ashed) Sheep D	0.48	48.7 ± 9.9	6
Rayden's values	0.68	54.6 ± 8.3	31 (7 sheep)

* $H = B + E(1 - E_A)$

but no observations were made, all results being collected during the final 4 days.

Results

The detailed results are given in Appendix Tables 1-6. They are considered below under the headings of energy retention, protein and fat retention, partition of the energy loss, and components of the heat production.

Energy retention

First a linear equation was used to describe the relationship between heat production per kg.$^{0.75}$ of body weight and metabolisable energy above energy equilibrium, giving the values of K_A and B (see Marston's equation on p. 46) shown in Table 5. The values of B (89.4 and 49.7 kcal./kg.$^{0.75}$/24 hr. for sheep 2 and 3 respectively) were different for the two sheep, and that for sheep 2 was considerably different from Marston's value (84.6 kcal./kg.$^{0.75}$/24 hr.). According to the linear hypothesis, B should be a constant quantity independent of the food and of the size of the animal. Although the number of values of M and E was smaller in the present experiment than in Marston's, higher food intakes were achieved. On the basis of these considerations it seemed reasonable to use another equation to describe the relationship between food consumption and energy exchange and to compare its usefulness with that of the linear equation.

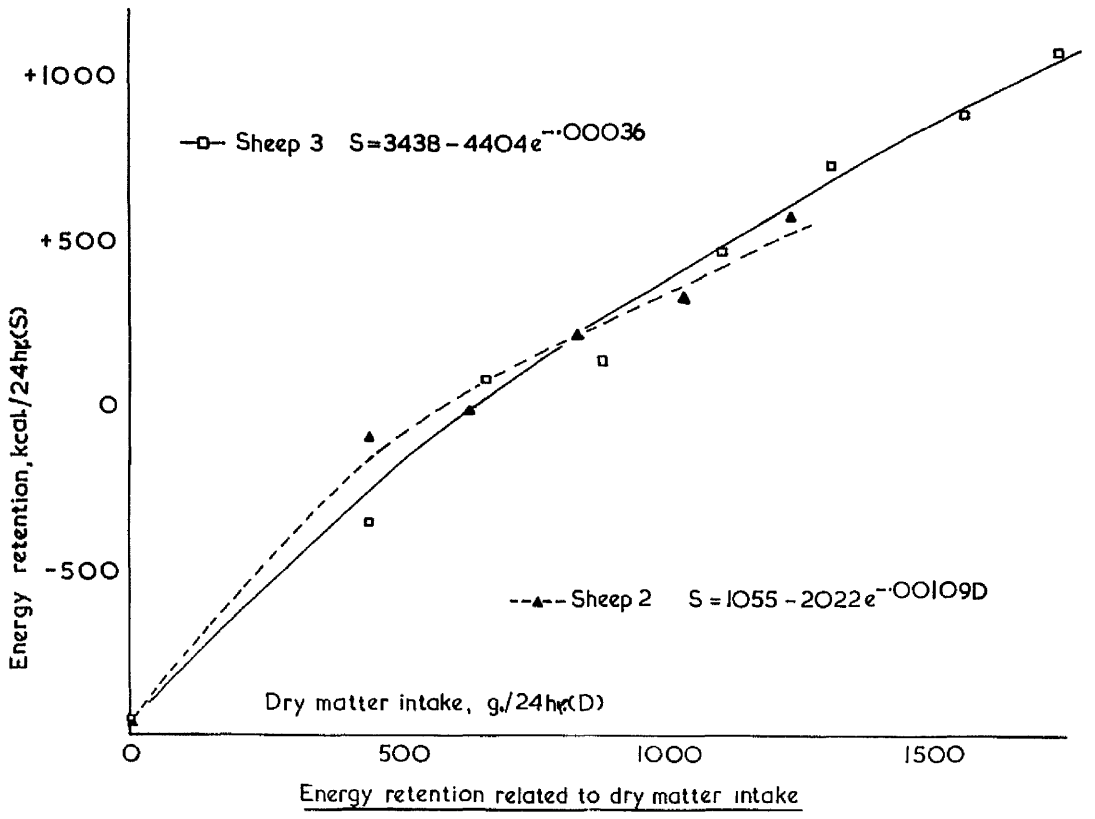

+1000
+500
0
-500
Energy retention, kcal./24hr(S)
Sheep 3 S=3438-4404e^-.00036
Sheep 2 S=1055-2022e^-.00109D
Dry matter intake, g./24hr(D)
0
500
1000
1500
Energy retention related to dry matter intake

Fig. 6.

Now a net energy value can be defined as the increase in energy retention which occurs per unit increase in food intake. In other words, it may be regarded as the slope of a curve relating energy retention to food intake. The relationship between food intake and energy storage for each sheep was described by means of exponential regressions fitted to the observed values, using the maximum likelihood solutions for the general equation

$$S = L - Be^{-kI}$$

where L, B and k are constants, I is the energy intake and S the energy retention (see example on p. A1 of the Appendix). The two equations obtained were:-

Sheep 2 . . . . S = $1055 - 2022e^{-2.4I \times 10^{-4}}$

Sheep 6 . . . . S = $3450 - 4404e^{-0.8I \times 10^{-4}}$

Linear equations fitted to the same data were:-

Sheep 2 . . . . S = 0.285I - 855

Sheep 6 . . . . S = 0.367I - 858

The data (in terms of dry matter intake) are plotted in Fig. 6. The linear equations underestimated the actual fasting metabolism of the sheep by about 100 kcal. in a total of about 1000 kcal. and an analysis of variance showed that, despite the small

Table 6. Energy retention in terms of protein and fat at different energy intakes

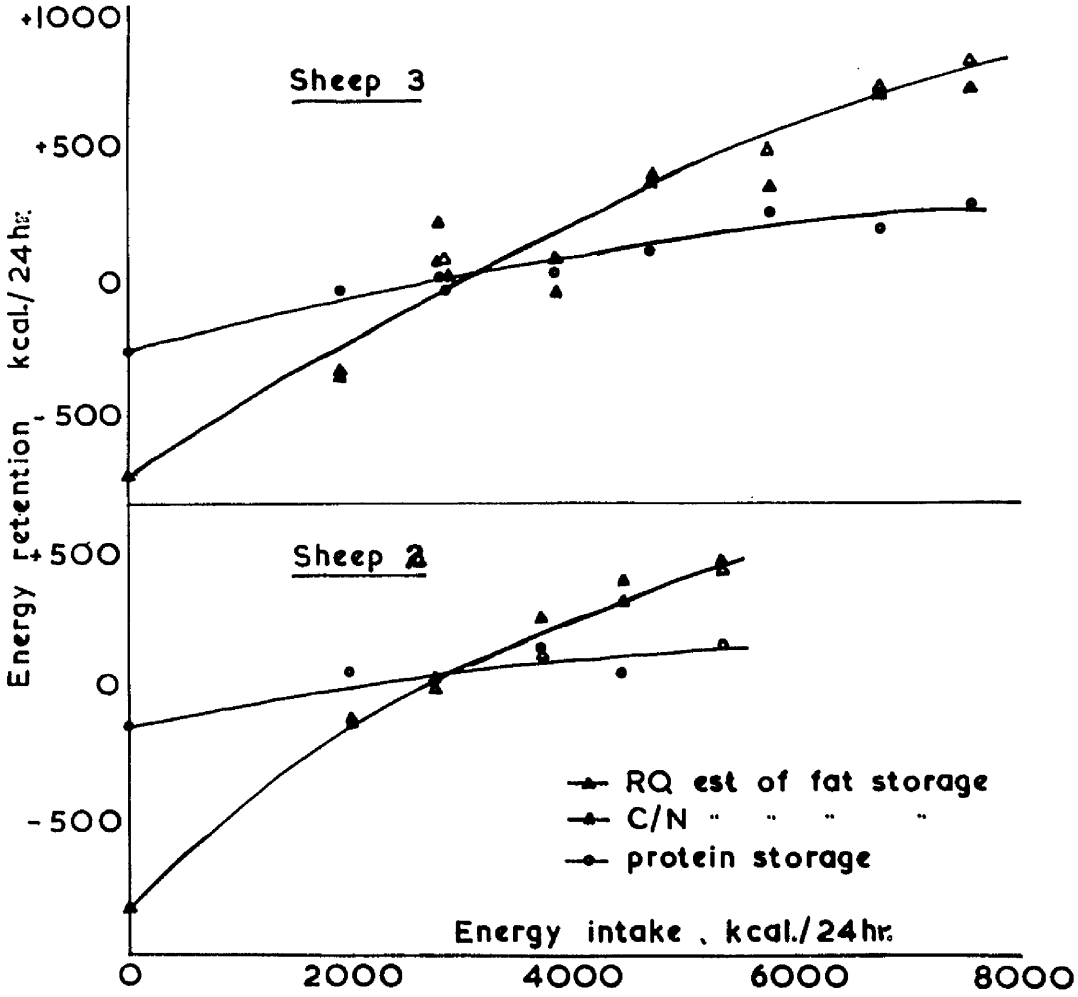
	Energy intake (kcal. per 24 hr.)	Energy retention (kcal. per 24 hr.) as:-		
		Protein	Fat	
			O/N estimate	R.Q. estimate
Sheep 8	0	-152	-	-832
	3084	59	-146	-128
	3787	8	31	- 15
	3714	140	255	184
	4401	62	304	336
	5860	166	468	459
Sheep 6	0	-264	-	-782
	1952	- 18	-344	-341
	2891	- 30	9	63
	2838	18	214	98
	3642	45	- 51	91
	4723	119	396	375
	5504	360	365	486
	6701	196	685	789
	7005	202	708	809

number of degrees of freedom available for the comparison, the inclusion of three rather than two constants in the regression resulted in a significant reduction of the residual error term: for the results from sheep 2 and 3 respectively, the odds were 10:1 and 14:1 against the reduction occurring by chance.

Protein and fat retention

As already mentioned (p. 39) energy retention or loss is mainly the resultant of changes in the amounts of fat and protein in the body, there being little storage of carbohydrate: this principle forms the basis of the C/N method of estimating energy retention.

In Table 6 the energy stored by each sheep in the form of protein and fat is given for each energy intake. The values were calculated as already shown in Table 5 (facing p. 40). An independent estimate of the fat storage was obtained as the difference between the total energy retention, estimated by the A.-L. method, and the energy retained as protein. It will be observed from Table 6 that considerable differences existed between the two members of some of the pairs of values for fat retention calculated in the two different ways, while the members of other pairs of values agree closely. Thus, for example, with sheep 3, at an energy intake of 2886 kcal. per 24 hr., the two values



Energy stored in fat & protein related to energy intake

Fig. 7.

Table 7. The percentage contributions made by protein, fat and ash plus water to the body weight changes of sheep 3 at several stages of nutrition

Mean body weight change (g. per 24 hr.)	Percentage contribution made by:-		
	Protein	Fat	Ash and water
- 80	5	40	51
+ 10	15	40	41
+176	5	6	89
+216	10	18	72
+272	13	19	68
+309	13	24	64
+378	20	32	48

were 214 and 78 kcal. per 24 hr., a fairly wide divergence, whereas for an energy intake of 4725 kcal. per 24 hr. they were as close as 596 and 573 kcal. per 24 hr. The fact is that estimates of fat storage are subject to a large error because they include so many terms. These results are also presented graphically in Fig. 7. The retentions of energy as both protein and fat showed a curvilinear relationship with energy intake, but the values for fat, which accounted for 70-90% of the total energy retained, extended over a much wider range than did the values for protein energy. Retention of energy as protein started at a lower energy intake than retention as fat. One reason for this in sheep is probably the large demand on protein for wool growth.

From the results given in Table 6 and from the changes that occurred in the body weights of the sheep, it was possible to calculate the contribution made by protein, fat and water plus ash to the total body weight gain or loss at various planes of nutrition. These values for sheep 5 are shown in Table 7 where it will be seen that protein contributed 6 to 20%, fat 6 to 46% and water plus ash, including changes in the gut contents, 41 to 89%. The table shows that, certainly with sheep, body weight changes in themselves cannot be regarded as reliable indications of the actual changes that are occurring in the tissues.

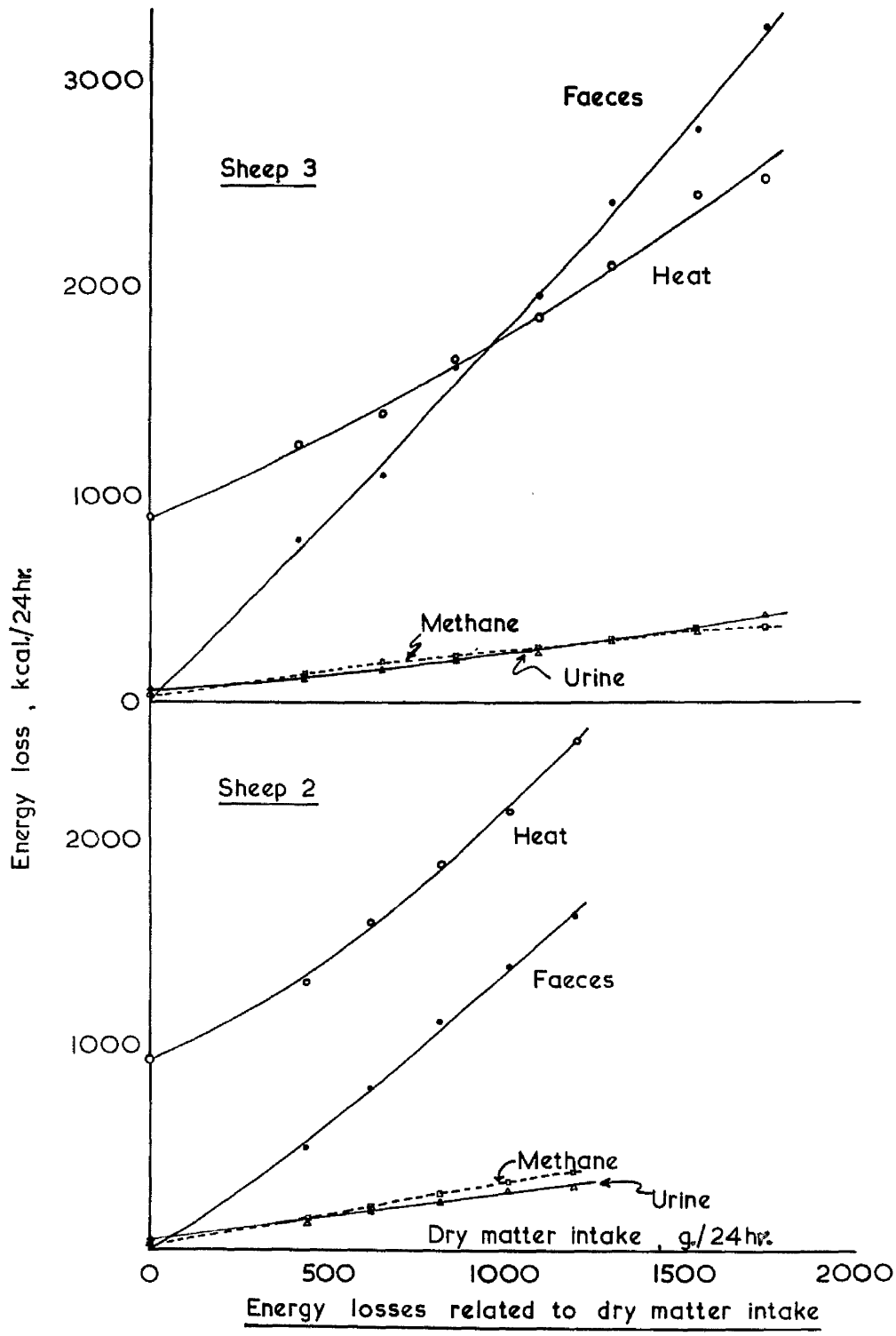

3000
2000
1000
0
Energy loss , kcal./24hr.
Sheep 3
Faeces
Heat
Methane
Urine
Sheep 2
2000
1000
Heat
Faeces
Methane
Urine
Dry matter intake , g./24hr.
0
500
1000
1500
2000
Energy losses related to dry matter intake

Fig. 8.

Partition of the energy loss

The separate components of the energy loss, i.e. the amounts lost as heat and in faeces, urine and methane, are plotted against dry matter intake for sheep 2 and 3 in Fig. 8. The equations for the curves for faecal, urinary and methane losses were:-

Sheep 2

$$F = I - 16858 - 168356e^{-4.6I}$$

$$U = 50 + 0.05I$$

$$M = 944 - 946e^{-9.5I}$$

Sheep 3

$$F = I - 24909 - 24989e^{-3.6I}$$

$$U = 75.96 + 35I$$

$$M = 1556 - 1312e^{-3.6I}$$

where $I \times 10^5$ = gross energy intake (kcal. per 24 hr.), and F, U and M are the faecal, urinary and methane energy in kcal. per 24 hr. The energy losses per 100 kcal. intake were obtained from the first differentials of these equations, and the heat production term by difference.

$$dH/kI = (1 - dR/dI) - (dF/dI + dU/dI + dM/dI)$$

where R is the energy retention. In Table 8 these differential terms are given for the maintenance level of feeding and for a level of feeding such as to give a 50% greater retention of energy. The table shows that the faecal losses in kcal. per 100 kcal. of intake increased with level of feeding while

Table 8. Partition of the energy losses at two planes of nutrition as calculated from the experiment in Exp. 2 (Expt. was 100 kcal. intake)

Level of feeding	Sheep no.	Grade	Faeces	Urine	Methane	Heat production++	Total loss of energy
Maintenance	2	B (chopped)	31.5	5.0	7.0	30.5	74.5
Above maintenance*			39.0	5.0	5.5	36.7	86.2
Maintenance	5	C (coarse cubes)	30.9	5.4	4.2	24.1	71.0
Above maintenance*			42.3	5.3	4.9	33.7	75.6

* The sheep were receiving 50% more net energy than required for maintenance.

++ Obtained by difference from intake less retention.

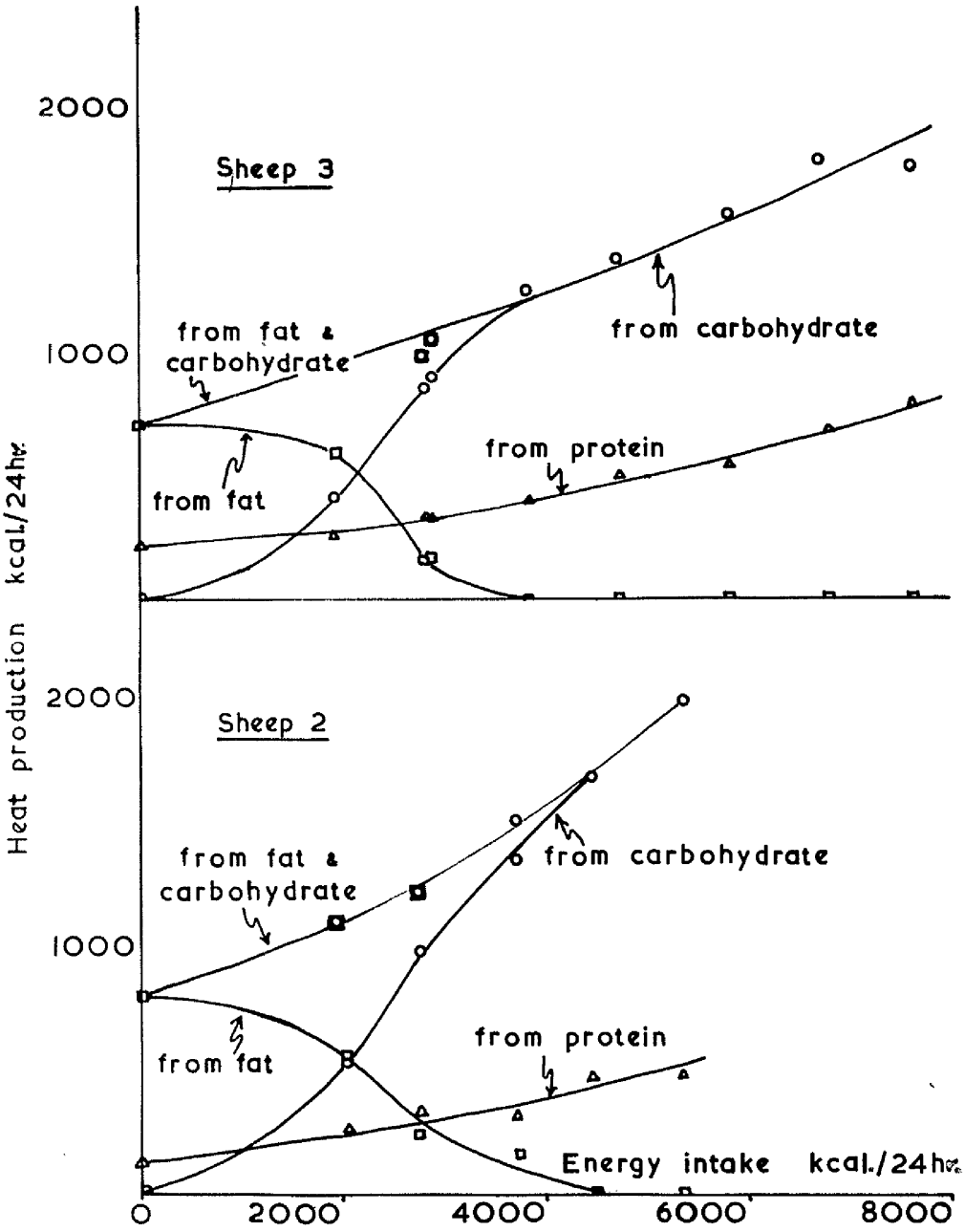
the corresponding methane losses decreased; urinary losses did not change in sheep 2 but increased in sheep 3; and the heat production either increased or decreased very slightly. At both levels of feeding the heat increment due to the cubed grass was considerably lower than that due to the chopped grass while the reverse was true of the increment of faecal energy loss. In spite of this the total increment of energy loss (and therefore the net energy value of the food) was practically the same for both grasses at the maintenance level (74.5 and 71.6 kcal. per 100 kcal. intake). As described below a more detailed consideration of the components of the heat production followed this observation.

Components of the heat production

The heat productions arising from the oxidation of protein, fat and carbohydrate are estimated together in the course of the calculation of the total heat production by the R.Q. method (see Table 5, opposite p. 40) but they are given separately and with the gross and absorbed (digested less methane) energy intakes in Table 9. To make the meaning of these figures clear, their origin may be described thus:

Heat of oxidation of protein (kcal.), H_p

= g. N in urine x 36.5



Components of the heat production related to energy intake

Fig. 9.

Table 9. Components of the heat production at different energy intakes (kcal. per 24 hr.)

	Gross energy intake	Absorbed energy intake	Non-protein R.Q. (corrected for methane production)	Heat from the oxidation of:-		
				Protein	Fat	Carbohydrate
Sheep 2	0	0	0.71	122	709	0
	2054	1605	0.80	305	502	556
	2737	1702	0.93	327	259	904
	3714	2857	0.97	304	104	1650
	4401	2754	1.03	471	0	1600
	5660	3657	1.00	478	0	1565
Sheep 3	0	0	0.67	204	691	0
	1951	1031	0.84	244	568	419
	2801	1615	0.90	346	152	696
	2858	1565	0.90	351	144	638
	3349	1960	1.00	356	0	1247
	4726	2815	1.02	470	0	1571
	5604	3157	1.06	556	0	1549
	6751	3594	1.00	575	0	1759
	7606	4021	1.11	706	0	1721

Heat of oxidation of fat (kcal.), H_f

$$= 3.33(1 - R.Q.)(H_T - H_p)$$

Heat of oxidation of carbohydrate (kcal.), H_c

$$= H_T - H_p - H_f - H_M$$

where H_T is the total heat production in kcal., H_M is the energy of the methane produced in kcal., and R.Q. is the non-protein respiratory quotient corrected to complete oxidation of methane as explained on p. 38.

The values given in Table 9 have been plotted against gross energy intake in Fig. 9, where it will be seen that the relationships between energy intake and the heat produced from the oxidation of protein, fat and carbohydrate were curvilinear. The heat production from carbohydrate rose rapidly as energy intake increased, whereas with protein the rise, though marked, was very much less. The heat production from fat was as high as 600 kcal. per 24 hr. at the fasting level but this value fell as energy intake increased until, with an intake of about 4000 kcal. per 24 hr., there appeared to be no net oxidation of fat. In other words, as the energy intake was increased from zero, the oxidation of body fat to meet the animal's basal requirements of energy was replaced by the oxidation of dietary carbohydrate. Additional carbohydrate dissimilation, above that required for this fat-sparing effect, must

Table 10. Components of the heat production below and above maintenance

Gross energy intakes (kcal. per 24 hr.)	Heat production, as a percentage of energy intake, that was due to oxidation of:-			
	Protein		Carbohydrate and fat	
	Sheep 2	Sheep 5	Sheep 2	Sheep 5
0 - 2000 (below maintenance)	4	4	15	14
2000 - 5000 (above maintenance)	9	9	15	27

also have taken place in order to supply the energy required for the synthetic activities promoted by the increasing food intake. Both sheep started to store fat at energy intakes of about 2600 kcal. per 24 hr. although, as already stated, some heat continued to be produced from the oxidation of fat until the intake was about 4000 kcal.

As shown in Table 10, which was derived from Fig. 9, between intakes of zero and 2000 kcal. per 24 hr. the heat produced from non-protein sources was 15% of the total energy intake for sheep 2, and 14% for sheep 5, while the corresponding values between intakes of 2000 and 5000 kcal. per 24 hr. were 15% and 87%, respectively. The heat produced from protein by both sheep was about 4% of the energy intake below maintenance and 9% above it. Since, at any one level of feeding, the digestibility of the two grasses differed considerably, it is useful to compare these heat increments of the grasses at corresponding levels of absorbed energy as well as at corresponding levels of gross energy intake. It was found that between absorbed energy intakes of 2000 and 5000 kcal. per 24 hr., the non-protein heat productions were 65% of the absorbed energy intake for sheep 2 on the chopped grass and 60% for sheep 5 on the cubed grass. Since the cubed grass had been ground finely before it was cubed and since

the grasses were very alike chemically, it seems likely that some of this difference of 15% between the heat increments of the two grasses at the same levels of absorbed energy is to be accounted for by the extra physical work involved in masticating and digesting the chopped grass. If all the 15% were to be accounted for in this way it would mean that the work done by the sheep in the comminution of 1000 g. of chopped grass would have given rise to the large amount of 400 kcal. of heat, the grass in this instance having a digestibility of 60% and a calorific value of 4.4 kcal. per g.

Discussion

The expression of net energy values

The use of an exponential equation rather than a linear one to describe the relationship between food intake (D) and energy retention (S) gives rise to several alternative measures of net energy value. It may be expressed as the differential of the exponential equation (NEV_d) or as the average slope of the curve between any two dry matter intakes, D_1 and D_2 (NEV_a). Thus,

since $S = I - Be^{-kD}$ (see p. 51),

$$NEV_d = dS/dD = kBe^{-kD}$$

and, $$NEV_a = 1/(D_2 - D_1) \int_{D_2}^{D_1} kBe^{-kD}.dD$$

Three methods of expressing net energy value used by earlier workers were, first that of Kellner (55) who made his measurements at maintenance and one higher level of feeding, secondly that of Armsby (5) who, in some experiments at any rate, used maintenance and one lower level and thirdly that of Forbes who in his earlier work (see ref. 60) used fasting together with a level of feeding sufficient to give a positive energy retention. These net energy values may be derived from the exponential equation to give:-

$$\text{Kellner's NEV} = B(e^{-kD_m} - e^{-kD_x})/(D_x - D_m)$$

$$\text{Armsby's NEV} = B(e^{-kD_x} - e^{-kD_m})/(D_m - D_x)$$

$$\text{Forbes' NEV} = B(1 - e^{-kD_x})/D_x$$

where D_m and D_x are the dry matter intakes at maintenance and at one other level, respectively.

Now if the relationship between energy retention and food intake is curvilinear, net energy values will decline with increasing food intake. The values determined by Armsby for hays fed below the maintenance level were, indeed, higher than those determined by Kellner for hays fed above maintenance. Many earlier workers used a linear relationship having different slopes above and below maintenance, and showed a marked difference in the net availability of dietary energy for maintenance (K_m) and for fattening (K_f). The ratio K_f/K_m has been given

values ranging from 0.70 to 0.85 (58, 76, 77): the values obtained here were 0.81 for sheep 2 and 0.75 for sheep 3.

Definition of plane of nutrition

Since the net energy value of a food declines as food intake increases, it is desirable to define plane of nutrition either in terms of the actual food intake or in terms of the energy retention promoted by the food. As pointed out by Brody (34), these two methods lead to quite different definitions.

Plane of nutrition may be expressed in terms of dry matter intake as,

$$P^{E} = (D/D_{B=0}) - 1,$$

where D is the dry matter intake at a plane of nutrition P^{E} and $D_{B=0}$ is the dry matter intake at the maintenance level of feeding. Thus the maintenance level of feeding is made the 'zero plane of nutrition'. However, if the curvature of the relationship between energy retention and food intake varies much from food to food, identical planes of nutrition for different foods would correspond to widely divergent levels of energy retention. The second definition avoids this feature while retaining the others by using,

$$P = B/B_{0}$$

where B is the energy retention at a plane of

nutrition P, and S_0 is the fasting energy loss or the maintenance requirement of net energy.

With regard to the relationship between plane of nutrition and net energy, as described on page 60,

$$NEV_d = kBe^{-kD}$$

therefore, using the first definition of plane of nutrition,

$$NEV_d = kBe^{-kD_{E=0}(P^E+1)}$$

Using the second definition leads to a different equation.

$$\text{since } S = L - Be^{-kD}$$

$$S_0 = B - L$$

$$\text{therefore } P = S/S_0$$

$$= (L - Be^{-kD})/(B - L)$$

which gives,

$$Be^{-kD} = P(L - B) + L$$

$$\text{therefore } NEV_d = kP(L - B) + kL$$

The differential net energy value (NEV_d) is thus related to P^E by an exponential equation but to P by a simple linear one. The definition of plane of nutrition in terms of energy retention (P) will be the one used in the remainder of this thesis.

Comparisons between animals of different size

To permit direct comparisons of energy

utilisation by animals of different sizes, that is animals with different basal energy losses, it is necessary to modify the terms of the exponential equation, $S = L - Be^{-kD}$, used to relate energy retention, S, to dry matter intake, D. This may be done by dividing the exponential equation throughout by the fasting energy loss, S_0. This gives

$$S/S_0 = L/S_0 - (B/S_0)e^{-kS_0(D/S_0)}$$

$$\text{or} \quad S^x = L^x - B^x e^{-k^x D^x}$$

where x signifies the modified terms which could be used for between-animal comparisons, S^x in fact being plane of nutrition (P) as defined in the previous section.

The differential net energy value (NEV_d), which was shown in the previous section to be related to the plane of nutrition (P) by a linear equation, is not altered by these modifications. Thus, as described on p. 58,

$$NEV_d = dS/dD = kBe^{-kD},$$

and using the modified terms,

$$dS^x/dD^x = k^x B^x e^{-k^x D^x}$$

$$= kS_0(B/S_0)e^{-kS_0(D/S_0)}$$

$$= kBe^{-kD}$$

$$= dS/dD.$$

$$\text{i.e.} \quad NEV_d = de^{x}/dD^{x}$$

The relationship between plane of nutrition and NEV_d also remains the same. Thus, as described on p. 61,

$$NEV_d = kP(L-B) + kL$$

and using the modified terms,

$$k^{x}P(L^{x} - B^{x}) + k^{x}L^{x} = km_0P(L/\theta_0 - B/\theta_0) + km_0L/\theta_0$$

$$= kP(L-B) + kL$$

$$\text{i.e.} \quad NEV_d = k^{x}P(L^{x}-B^{x}) + k^{x}L^{x}$$

It follows from this that the actual food intakes and energy retentions of animals of different sizes may be expressed as multiples of their fasting energy losses (requirements of net energy for maintenance) for comparative purposes without introducing any error in the calculation of the net energy value of the food or in the relationship between net energy and plane of nutrition.

To conclude this discussion of food evaluation it may be said that since the results of the present experiment tend to confirm the existence of a curvilinear relationship between food intake and energy retention and to deny the applicability of a linear

hypothesis such as that of Marston, the important problem is the mathematical description of this curvilinearity. The solution adopted by Forbes & Swift (41) and supported by Marston (70) was to separate the processes of energy retention below maintenance from those above and, since farmers are mainly interested in production, to assume linearity of the relationship above maintenance. However, the present results suggest that even above maintenance linearity may not be assumed. Both Hellberg (60) and Mitchell (75) have, in effect, used quadratic functions to fit their data while an exponential equation has been used here and by Wagner & Ghoneim (97). It is difficult to decide between the two functions on statistical grounds but the exponential form is to be preferred for its ease of manipulation.

Summary

1. The object of the experiment described in this chapter was to elucidate the relationship between food intake and energy exchange.

2. The energy exchanges of two sheep were measured. One of them was fed with chopped dried grass at five different levels and the other with dried grass cubes at seven different levels. The fasting energy losses of the sheep were also measured.

3. From the results it was concluded that, over a wide range of food intakes, an exponential

description of the relationship between energy retention and food intake fitted the facts better than a linear one.

4. The exponential equation used for this purpose was adapted to the calculation of net energy values, to the definition of plane of nutrition and to the modification of the actual energy exchange of an animal to permit comparisons between animals of different sizes.

5. Of the body weight changes at the various levels of feeding, 8 to 20% was accounted for by protein, 6 to 46% by fat and 41 to 89% by water plus inorganic material (including changes in gut contents).

6. For each unit of food eaten, the amount of energy lost in faeces, urine, methane and as heat varied with the level of feeding. These changes were described mathematically.

7. While the loss of energy in the faeces was higher with cubed grass than with chopped grass, the increase in heat production due to feeding the cubed grass was lower than that due to feeding the chopped grass. These differences have been examined further in the experiment described in the next chapter.

Chapter 3

The effect of the physical form of the food on energy utilisation (Exp. (5))

Because the second experiment, described in the previous chapter, showed differences between the utilisation of the energy of cubed and chopped grass, the present experiment (Exp. (5)) was designed to provide more critical information on the subject.

Forbes, Fries and Braman (37), using a steer, found that the net energy value of a sample of alfalfa hay was the same whether the hay was chopped or ground into a fine meal. The dry matter of the chopped hay was about 2% more digestible but this was balanced by the heat production being about 3% higher. Their results showed that the digestibility of the crude fibre fraction was most affected by the grinding process. More recently it has been reported that milk production was higher when cows were given part of their production requirements as pelleted rather than chopped alfalfa hay (28). It has also been found that the pelleting of timothy meal for lambs increased the daily gains of weight but that there was no such effect with lucerne or maize meals (30). In both these last publications the issue was confused by variations in the total amounts of food eaten by the experimental animals. The authors referred to a number of American reports of variable effects on

Table 11. Particle size distribution for mass A

Sieve opening (mm.)	% passing through		
	Chopped material	Coarse cubes	Fine cubes
0.16	1	23	25
0.35	4	39	39
0.50	15	38	36
retained by 0.50 sieve	80	9	2

the body gain of ruminants due to the pelleting of feedingstuffs. Yet other workers have reported that although the pelleting of calf "starter" meal did not affect the efficiency of food conversion it resulted in higher food intake (65).

In the present experiment sheep were used to compare the utilization of the energy of chopped grass with that of two grades of cubes, at two levels of dry matter intake (18).

Experimental

One third of the dried grass used (D) was chopped coarsely, one third was ground to pass through a coarse sieve (1.8 meshes per cm.) and cubed, and the remainder was ground to pass through a fine sieve (5 meshes per cm.) and then cubed. Some details of the physical state of these materials are given in Table 11, and the chemical composition is shown in Appendix Table 1. A further quantity of the same material was used in the rate of passage studies reported elsewhere (80).

Three sheep (nos. 14, 15 and 16) were given 1500 g. of dried grass per day: in the chopped form in the first period, as coarse cubes in the second and as fine cubes in the third. Another three sheep (nos. 17, 18 and 19) were given 600 g. per day in the same way. The daily ration was given in equal amounts at 10 a.m. and 5 p.m. Each period lasted for 5 weeks, the sheep being confined in the

respiration chambers and their excreta collected during the last 6 days. The respiratory exchange of the sheep was measured on the last 4 days of each period. Chemical analyses were done on composite samples of excreta, each representing 2 days' excretion.

Finally, the energy exchange was measured on the third and fourth days of starvation to enable exponential equations to be used to describe the relationships between food intake and energy retention.

Results

Slight differences between the energy intakes of the sheep at each level of feeding arose from two causes. First, the dried grass was weighed on an air-dry basis but the cubes had a higher dry matter content than the chopped material. Secondly, small amounts of the chopped grass were refused at the higher level of feeding.

The complete results are given in Appendix Tables 1-6.

Energy retention and food intake

To describe the energy utilization fully, exponential equations were fitted to the energy intake/retention data for each ration. The general equation was,

$$S = L - Be^{-kI},$$

where S is the energy retention, I is the energy intake and L, B and K are constants.

Now when $I = 0$,

$$S = S_0 = L - B$$

therefore,

$$S - S_0 = B(1 - e^{-kI})$$

thus $$\frac{S_2 - S_0}{S_1 - S_0} = \frac{1 - e^{-kI_2}}{1 - e^{-kI_1}}$$

where the subscripts 0, 1 and 2 refer to zero, low and high food intakes, respectively. From this last equation the values of k were obtained by successive approximations, and B was then calculated as

$$B = \frac{S - S_0}{1 - e^{-kI}}$$

Finally, L was obtained as

$$L = B + S_0$$

Since the fasting energy expenditures of the six sheep were virtually the same, all the values were pooled before making the above calculations. The results of the calculations are given in Table 18, where it will be seen that the constants for the

Table 12. The constants in the exponential equation* used to relate energy retention (E) to energy intake (I)

Ration	E_r	B	k
Group D — Chopped	1364	2800	2.0×10^{-4}
Group D — Coarse cubes	1533	3579	3.5×10^{-4}
Group D — Fine cubes	1670	2805	3.0×10^{-4}
Group B — Chopped (Exp. (2))	1065	6909	2.4×10^{-4}
Group C — Coarse cubes (Exp. (3))	3457	4024	0.0×10^{-4}

* $E = I_r - Be^{-kI}$

three rations were similar one to another and to those obtained for grass B in Exp.(2); the constants for grass C were somewhat different, indicating a more nearly linear relationship between energy retention and energy intake for that grass.

From these exponential equations it was shown that the sheep required 484, 494 and 471 g. dry matter per day in the form of chopped grass, medium cubes and fine cubes, respectively, to maintain energy equilibrium ($S = 0$ and $L = Se^{-kI}$). At a plane of nutrition of 0.75 (i.e. at a positive energy retention of 760 kcal. per 24 hr., 75% higher than the net energy requirement for maintenance, see p. 60), the dry matter requirements were 1291, 1155 and 1157 g. per day, indicating a slight superiority of the cubed grass over the chopped grass.

Thus, at planes of nutrition (P) near the maintenance level ($P = 0$) the cubed and chopped grass were of equal value but at higher intakes, e.g. $P = 0.75$, the cubes were slightly better.

Partition of the energy loss

The amounts of energy lost through the different pathways, when the sheep were receiving the various rations, were compared. First the faecal, urinary, gaseous and heat losses (in excess of the fasting values) were expressed as percentages of the gross energy intake in order to eliminate the small effects

Table 13. Energy exchange recorded by Mean D, expressed as kcal. per 100 kcal. of gross energy intake (means for 5 sheep)

Intake	Component of energy exchange	Chopped	Coarse cubes	Fine cubes	Standard error (±)
High	Faecal loss	26.0	34.0	37.9	1.1
	Urinary loss	5.3	4.9	6.0	0.4
	Methane loss	7.6	5.9	4.6	0.5
	Heat increment	28.0	31.3	31.9	0.5
	Net energy	33.5	33.3	30.3	1.0
Low	Faecal loss	38.5	39.6	38.1	1.1
	Urinary loss	5.1	4.3	4.7	0.4
	Methane loss	6.2	7.4	7.8	0.5
	Heat increment	17.6	13.5	13.4	0.5
	Net energy	48.6	45.3	46.0	1.0

Table 14. Statistical significance of the differences found in various comparisons with cross 3

Comparison	Component of energy exchange				
	Faecal loss	Urinary loss	Methane loss	Heat increment	Net energy
High v. low intake	VHS	NS	VHS	VHS	VHS
Chopped v. coarse or fine cubes	VHS	HS	VHS	VHS	HS
Between sheep	HS	S	HS	S	HS

VHS = significant difference at the 0.1% level of probability.

HS = significant difference at the 1% level of probability.

S = significant difference at the 5% level of probability.

NS = difference not significant at the 5% level of probability.

due to the slight differences in energy intake at each level of feeding, mentioned on p. 68. These results are given in Table 13 together with the net energy expressed similarly. An analysis of variance of the pooled results for each energy category gave the standard errors of the means that are included in Table 13. The statistical significance of the differences between the three rations and between the two levels of intake that appear in Table 13 is shown in Table 14.

The data in these tables show that, for a given energy intake, the percentage of the energy lost in the faeces was considerably greater when the sheep were given cubed grass than when they were given chopped material. With all three materials the faecal losses were greater at the higher level of feeding. Methane production, on the other hand, showed exactly opposite effects.

The rate of passage experiment made with another sample of this same grass and reported elsewhere (30) produced parallel trends relating to losses of dry matter in the faeces. From the results obtained in that experiment it was concluded that the physical form of the food and the amount given modified its time of passage through the gut, and that this was the factor responsible for the wide variation in the digestibility of the dry matter between the different rations and levels of

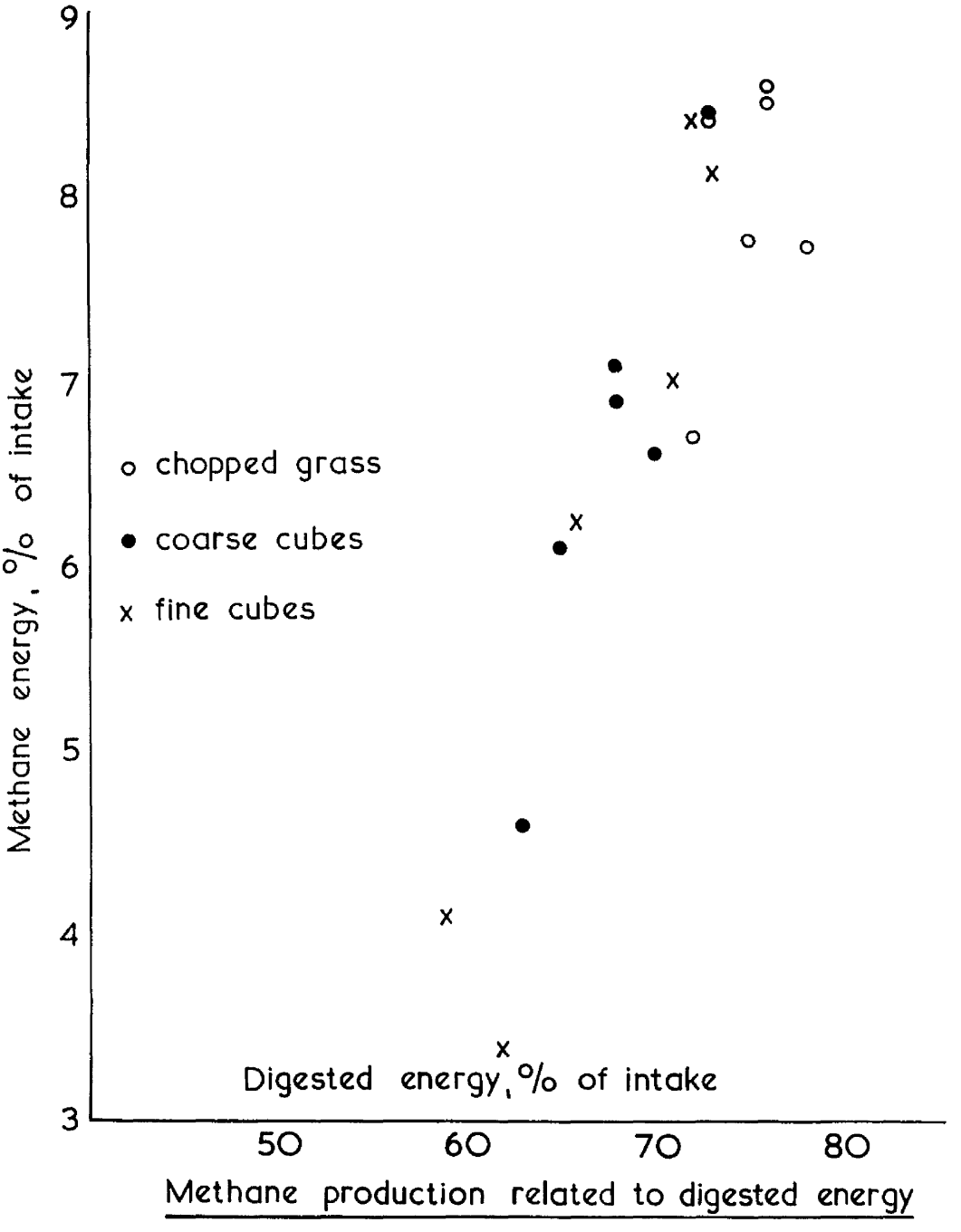

9
8
7
6
5
4
3
Methane energy, % of intake
o chopped grass
● coarse cubes
x fine cubes
Digested energy, % of intake
50
60
70
80
Methane production related to digested energy

Fig. 10.

feeding. The same factor was presumably involved in the present calorimetric trials. Thus the lower methane losses and higher faecal losses when cubes were given must have resulted from a shorter period of digestion. The fact that methane production was so markedly affected, particularly at the high level of feeding, supports the hypothesis that the emptying of the rumen, where fermentation is most active, is the main factor determining rate of passage. The differences in methane production between the sheep were marked (as shown in Table 14) and may have resulted from differences in rumen flora or in rumen size. Although the differences in digestibility of dietary energy between the sheep on any one ration were not statistically significant, as digestible energy increased methane losses also tended to increase, as shown in Fig. 10.

Table 15 shows that urinary losses of energy were possibly slightly less for the cubed grasses than for the chopped grass but the differences were not statistically significant.

As in Exp. (2), heat losses were considerably greater for chopped grass than for cubes whether the losses were expressed per unit of gross energy or of digested energy. Two points may be important in this connection. First, the sheep were never observed to ruminate while on the cubed diet though they spent much of their time doing so while on the

Table 15. Mean values for digestible, metabolizable and net energy of rows B at the high level of feeding (kcal. per 100 g. dry matter)

Energy category	Chopped	Sparse cubes	Fine cubes	Standard error (±)
Digestible	305	337	330	6
Metabolizable	266	240	240	6
Net	189	146	142	4

diet of chopped grass. Secondly, it took the sheep much longer to eat a meal of chopped fodder than one of cubes, especially at the higher level of intake. Thus a sheep given chopped material expended considerably more muscular energy in prehending, chewing and cudding than one fed on cubes.

The fact that the net energy values of the three rations were almost equal means that the additional digestible energy obtained from the chopped fodder was almost counterbalanced by an increase in energy lost as methane and heat, as was found by Forbes, Fries & Braman (37).

In Table 15 the digestible, metabolizable and net energies of the three rations are given for the higher level of feeding. If the value of these rations had been based on either the digestible or metabolisable energy, their real relative value, as given by the net energy, would have been obscured. Thus digestible energy would rate the value of the fine cubes as being some 30% below that of the chopped grass, and metabolizable energy would assess the value as being about 20% below. On the other hand, according to the figures for net energy all three materials were of equal value, the 5% superiority of the course cubes over the chopped grass not being statistically significant. Thus although these rations were identical chemically, the digestible and metabolizable energy grossly underestimated the

Table 16. The mean digestibilities of the chemical constituents of Trial 3

Constituent	High intake			Low intake			Standard error (±)
	Chopped	Coarse cubes	Fine cubes	Chopped	Coarse cubes	Fine cubes	
Dry matter	80.6	73.1	73.1	75.5	67.7	64.3	0.9
Organic matter	82.7	76.4	76.2	77.6	69.6	68.8	0.9
Ether extract	66.1	64.6	67.6	57.3	71.8	61.4	2.9
Crude fibre	58.9	74.7	67.7	76.5	56.1	49.9	1.6
Nitrogen-free extract	85.8	81.8	82.9	84.9	76.7	75.8	1.2
Crude protein	66.9	61.9	63.6	61.9	58.7	54.8	1.4
Cellulose	67.5	60.6	70.6	81.4	62.5	56.4	1.7
Cellulosic pentosan	87.7	78.8	78.7	77.7	68.5	45.8	1.3
Non-cellulosic pentosan	82.5	75.9	74.9	76.9	66.4	60.1	3.6

Table 17. Statistical significance of the differences found in various comparisons with cross 2

Digestibility of:-	Comparison		
	High v. low intake	Chopped v. coarse or fine cubes	Between sheep
Dry matter	VHS	VHS	NS
Organic matter	VHS	VHS	NS
Ether extract	NS	NS	NS
Crude protein	HS	S	NS
Crude fibre	VHS	VHS	NS
Nitrogen-free extractives	NS	NS	NS
Cellulose	VHS	VHS	NS
Cellulosic pentosan	VHS	VHS	S
Non-cellulosic pentosan	NS	NS	NS

Symbols as in Table 14.

value of the cubes.

Digestibility of the chemical constituents of the grass

The complete digestibility results are given in Appendix Table 5 and are summarised here in Table 16. The standard errors and the statistical significance of the differences that occurred (given in Table 17) were obtained from analysis of variance of the pooled results. The results show that the amount and physical form of the food eaten had a marked effect on the digestibility of all the main groups of chemical constituents with the exception of the other extractives, for which there was, in any case, a much larger analytical error. The maximum depression of digestibility occurred in the crude fibre fraction and in the cellulose and non-cellulosic pentosan.

By grouping the cell wall constituents together and apart from the other organic constituents, it was calculated that the digestibility of the cell wall constituents at the low level of feeding was 81% for the chopped grass compared with 58% for the fine cubes: the corresponding figures for the other organic constituents were 79 and 76%. In other words, variation in the rate of passage of the food through the digestive tract markedly affected the cell wall digestibility but had only a relatively small effect on the digestibility of the cell contents. These results agree with those of Forbes et al. (37)

whose experiments were made with a steer, and are what might be expected since the fermentative digestion of the structural components of grass must be a relatively slow process and its extent must vary noticeably with the time allowed for it to occur.

Discussion

The results of this experiment further confirm that net energy is depressed by an increase in food intake with both cubed and chopped grass. An increased rate of passage through the rumen would account for the decrease in methane production which occurred when cubes replaced chopped fodder since most of the methane arises from the fermentation in the rumen. At the same time, larger quantities of soluble carbohydrates may escape fermentation in the rumen and be more efficiently digested lower in the digestive tract when the ration is cubed rather than chopped. Thus the amount of food eaten and its physical form may well affect the extent and nature of the fermentation in the rumen and possibly, therefore, the heat production arising from the digestion of the end-products.

The heat increments due to the oxidation of protein, carbohydrate and fat were calculated as in Exp. (2) between levels of 2000 and 3000 kcal. per 24 hr. of absorbed energy. The non-protein heat increment contains components due to fermentation in the rumen, to utilisation of the end-products of digestion and to physical work

of digestion. If it is assumed that with a single food given in different forms equal rates of passage through the digestive tract result in equal amounts of energy being absorbed as fatty acids and as sugars, then the difference between the non-protein heat increments of the chopped and cubed grass at the same levels of absorbed energy may be taken as due to differences in physical work of digestion. The non-protein heat increments were 30, 34 and 40 kcal./100 kcal. of absorbed energy for the chopped grass, medium cubes and fine cubes, respectively. Thus the physical work of digesting the chopped grass may have accounted for up to 10 to 16 kcal./100 kcal. of absorbed energy, that is 17 to 27% of the total heat production: this is equivalent to 35 to 40 kcal./100 g. of dry matter ingested, the dry matter having a calorific value of 4.4 kcal./g. and 60% of the grass energy being absorbed. Although these figures are very approximate and should not have too much importance placed on them, they agree reasonably well with those obtained in Exp. (3) and are large enough to suggest that more experiments ought to be made to determine accurately the energy cost of eating and chewing chopped grass.

Although the net energy value of the particular grass used in this experiment was not much affected by cubing, this may not be so with a very mature grass. While the rate of passage of such a grass

would probably be very slow when given in the chopped form, the passage of cubed material might well be as rapid as with the present grass. If this were so the effects of the wider difference in the extent of fermentation in the rumen might be sufficient to affect the net energy value.

Summary

1. The experiment described in this chapter was designed to determine the effects of the physical form of feed on the utilisation of its energy and on the digestibility of its constituents.

2. Three sheep were given in succession 600 g. per day of chopped, coarsely ground-and-cubed and finely ground-and-cubed dried grass. Another three sheep were given the same materials at the rate of 1500 g. per day. The energy exchange of the animals was measured on these rations and at the fasting level, and the digestibility of the chemical constituents of the rations was determined.

3. As found in Exp. (2), the net energy value of both chopped and cubed grass declined with increasing food intake.

4. At equal, low energy intakes the heat production of the sheep was considerably lower on a diet of cubed grass than when chopped grass was fed and the faecal energy loss was higher but the

net energy of the cubes was slightly greater than that of the chopped material.

5. The main cause of the higher faecal energy loss with cubes was a depression of the digestibility of the cell wall constituents. This depression in digestibility was related to a more rapid rate of passage of the grass given in the cubed form through the digestive tract.

6. When energy was the criterion on which food value was assessed, measures other than net energy gave misleading results.

7. The considerable difference between the heat increments of the chopped and cubed grass suggested that the energy cost of the physical work of digestion may be quite large.

Chapter 4

The effect of frequency of feeding on energy utilisation (Exp. (4))

It has been shown by Gordon & Tribe (45) that sheep have slightly better body weight gains per kg. of food eaten when they are given eight small meals per day than when they are given one large one, and these authors suggested that changes in the rate of passage of the food through the rumen might be the main cause. The study of rate of passage of food through the digestive tract by Blaxter, Graham & Wainman (20) did indeed indicate that the size and frequency of meals might have some effect on the digestion of the food.

From a practical viewpoint it would be of considerable value if the frequency of feeding could be reduced without incurring a serious loss in the efficiency of food utilisation, and for experimental purposes it is important to know whether the number of meals given per day should be standardised.

The present experiment (Exp. (4)) was designed to show whether, at a constant mean intake of food per day, frequency of feeding had any effect on the energy exchange of sheep.

Experimental

A medium quality chopped dried grass (E) was used. Its chemical composition is given in Appendix

Table 1.

Three sheep were used to compare the effects of three methods of feeding, which consisted of giving the whole daily ration (a) in one meal at 11.30 a.m., (b) in two meals at 11.30 a.m. and 11.30 p.m. and (c) in four meals at 11.30 a.m., 5.30 p.m., 11.30 p.m. and 5.30 a.m.

When the sheep were given 1500 g. or more of the grass in one meal they required about 24 hr. to consume it all. The choice of the level of feeding to be used for the above-mentioned comparisons was therefore determined by the maximum amount of food that the sheep would consume in the reasonably short time of 8 hr. This was found to be 1100 g. Accordingly each sheep was given, in successive periods, one meal of 1100 g., two meals of 550 g. and four meals of 275 g. as described, the sequence of treatments being different for each sheep.

Each period lasted for 28 days. Faeces and urine were collected on the last 6 days of each period and respiratory exchange was measured on the last 4 days. The methane production of each sheep was measured every 2 hr. throughout one whole day when they were receiving one and four meals per day. The excreta from each period were pooled into single analytical samples representing 6 days' collection, the faeces, which were of a tough consistency, being

Table 10. Energy exchanges recorded by means of, expressed as kcal./100 kcal. of gross energy intake (means for 5 sheep), when the sheep were fed once, twice and four times per day at a high level of intake and twice per day at a low level

Intake	Component	Meals per day: 1	2	4	Standard error of means ±	Statistical significance of differences
High	Faecal loss	26.4	28.1	28.2	0.6	NS
	Urinary loss	3.7	3.9	4.1	0.2	NS
	Methane loss	6.7	7.4	7.4	0.1	*
	Heat increment	22.9	25.2	21.8	1.4	NS
	Net energy	40.3	37.4	38.5	1.8	NS
Low	Faecal loss		28.2			
	Urinary loss		3.8			
	Methane loss		6.9			
	Heat increment		21.7			
	Net energy		40.0			

* Difference between treatments significant at the 1% level of probability.

NS Difference between treatments not significant at the 5% level of probability.

mixed with sufficient water to give a fluid suspension.

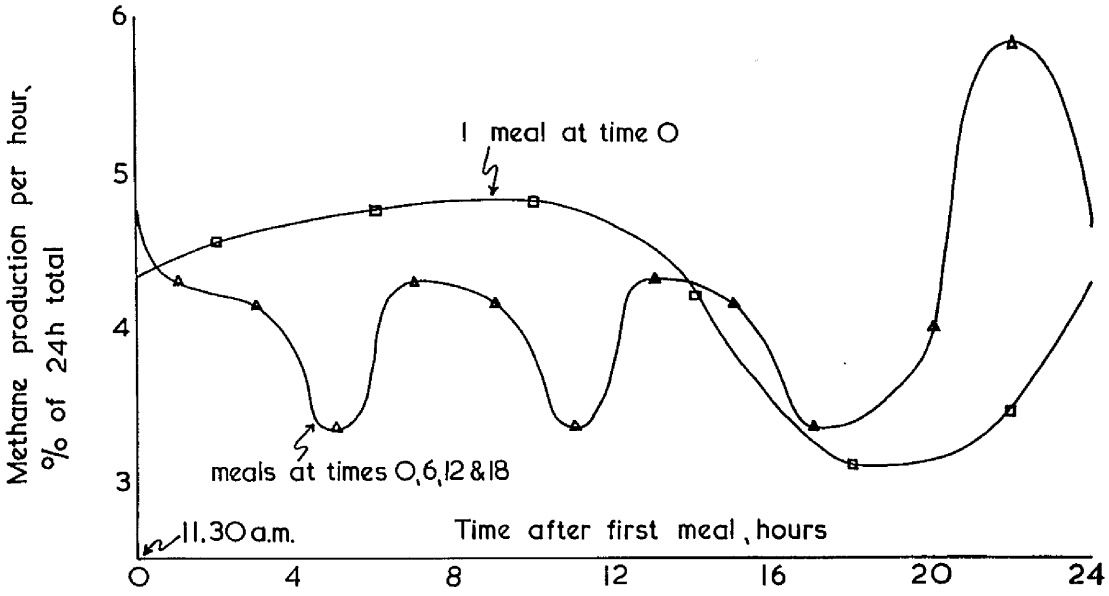
To provide data from which the net energy value of the feed could be calculated, the energy losses of each sheep on the third and fourth days of fasting and their energy retention when the level of feeding was 900 g. per day were determined after the three main treatments had been given.

Results and Discussion

The detailed results are given in Appendix Tables 1-7, and the main results are summarised in Table 16 and Fig. 11.

Statistical analysis

The data, expressed as kcal./100 kcal. of energy intake for the same reason as in Exp. (3), p. 70, were analysed to determine whether frequency of feeding had a statistically significant effect on the faecal, urinary and methane energy losses, on the heat increment and on the net energy. The statistical analysis showed that the only significant effect was on methane production (Table 18) which was about 10% lower when the sheep received only one meal per day than it was when they received two or four meals per day. There were quite large differences in faecal energy loss between animals, which may have obscured small trends related to these changes in methane production. The rather high standard error



Rates of methane production after feeding, (means of 3 sheep)

Fig. 11.

of the mean heat increments (21.4 kcal./100 kcal. energy intake, compared with 40.5 in Exp. (5) was probably due to the restlessness of the sheep used in the present experiment.

The rate of methane production over 24 hr.

The rate of methane production (per 3 hr.) was measured during 1 day when the sheep were receiving one large meal at 11.30 a.m. and also when they were receiving four small meals at 11.30 a.m., 5.30 p.m., 11.30 p.m. and 5.30 a.m. The detailed results, which are given in Appendix Table 7, have been summarised and presented graphically in Fig. 11 where rate of methane production is plotted against time. Inspection of the detailed results showed that, irrespective of the feeding pattern, the rate of methane production increased between 7.30 and 9.30 a.m. (except in one instance). Some observations suggested that this rise coincided with the recommencement of rumination in the morning but it may have been caused partly by a release of rumen gases due to the excitement at the opening of the respiration chamber at or just before 9.30 a.m. The figures plotted in Fig. 11 for sheep receiving one meal per day are the mean values of methane production per 3 hr. for the three sheep: for the sheep receiving four meals per day at intervals of 6 hr., all the values of methane production obtained

at 2, 4 and 6 hr. after a meal between 11.30 a.m. on one day and 7.30 a.m. on the following day were averaged to show the effect of giving a small meal, and the corresponding values obtained between 7.30 a.m. and 11.30 a.m. were averaged to show the early morning rise of methane production. Fig. 11 shows that there was a more uniform rate of methane production over most of the day when the sheep were fed several times per day rather than once. The changes which occurred between 11.30 a.m. on one day and 7.30 a.m. on the following day were probably related to changes in the amount of dry matter undergoing fermentation in the rumen at any particular time (30, 38).

Food intake and energy retention

Since there were no significant differences in net energy as a result of feeding in the different ways described (Table 13), the data were pooled and an exponential equation was used to relate energy retention (S) to energy intake (I) by the method explained on p. 69. The equation was

$$S = 3878 - 4166e^{-1.251I \times 10^{-4}},$$

This equation shows that, as in the previous experiments, the efficiency of energy utilisation fell considerably as the level of feeding was increased. Thus the net energy value of the food

was 171 kcal. per 100 g. of dry matter at a level of feeding below maintenance (Armsby's NEV, p. 59) and 150 kcal. per 100 g. when the level of feeding was above maintenance (Kellner's NEV, p. 59).

Summary

1. The experiment described in this chapter was designed to show whether, at a particular level of feeding, the energy exchange of sheep was dependent on frequency of feeding.
2. When the level of feeding was 1100 g. of chopped dried grass per day, the fact that the daily ration was divided into one, two or four meals per day did not significantly affect the energy retention of three sheep.
3. Although the frequent ingestion of small meals caused a more uniform rate of methane production over most of the day, the total methane production per 24 hr. was then higher than when a single large meal was given. This was probably due to a more rapid passage of some of the food from the rumen following the consumption of one large meal.
4. Measurement of the fasting energy losses of the sheep and of their energy retention when they were given 900 g. per day of the chopped dried grass, taken along with the other data, allowed energy retention to be related to energy intake

by an exponential equation. This showed that, as in Expe. (2) and (3), the efficiency of energy utilization was less at a high than at a low level of feeding.

Part III

Discussion

Chapter 4

Net energy values of the grasses

In this chapter the net energy values of grasses B, C, D and E which were calculated from the energy exchange data of Expe. (3), (5) and (4) are compared with the values computed from the digestibility data by the methods of Armsby (5) and Kellner (33).

The use of energy retention to obtain a measure of the nutritive value of food was introduced about the year 1900 by Armsby and Kellner who were working independently of each other. They measured the increment of energy retention promoted by the addition of a quantity of the test food to a basic ration which was sufficient either to maintain energy equilibrium (Kellner) or to allow a small loss of energy from the body (Armsby). No account was taken of possible effects on the digestion of the basic ration due to the addition of the test food.

The mean net energy values (for fattening steers) of the digestible organic matter in starch, molasses, wheat gluten, straw pulp and arachis oil (33) were used by Kellner to calculate the net energy values of digestible starch, nitrogen-free extract, protein, crude fibre and ether extract. He then compared the directly-measured net energy values for a series of foods with the values computed

Table 19. Kellner's factors for estimating the net energy value of roughages

Pure nutrients	Net energy value of "pure nutrients" (kcal. per 100 g. digested organic matter)
Starch	238
Crude fibre and nitrogen-free extract	170
Crude protein	234
Ether extract	450

When food is in long form deduct
1.30 net kcal. per g. crude fibre in 100 g. of the food

When food is finely chopped deduct
0.70 net kcal. per g. crude fibre in 100 g. of the food

Table 20. The experimental basis of Kellner's factors

No. of experiments	Substance added to a basic ration	Net energy value of the addition (kcal. per 100 g. digested organic matter)
16	Potato starch	172-352
3	Molasses	170-245
5	Wheat gluten	102-237
3	Arachis oil	372-459
3	Straw pulp	191-215

by using the factors he had obtained for the "pure nutrients". Because the predicted net energies were too high by amounts which, with about ten roughages, varied almost linearly with the crude fibre content of the food, he reduced the predicted values (kcal. per 100 g. of food) by 1.86 kcal. per g. of crude fibre in 100 g. of food (55). These factors are given in Tables 19 and 20 which also show the large amount of variation in the values making up each mean factor and the rather small number of experiments on which the system was based.

Armsby evolved a different method of prediction (5). Using Kellner's results as well as his own, also with steers, he calculated for various foods the metabolizable energy per g. of digested organic matter and the heat increment per g. of dry matter ingested by the experimental animals. It is not clear whether he intended these factors to be used in a general sense to compute net energy values or only within the narrow classes of food for which they had actually been measured, but he listed 183 net energy values computed from digestibility data, each one written to the second decimal place. The mean factors for roughages and the variation in their individual components are given in Table 21.

As was shown in Chapters 2, 3 and 4 of Part II of the thesis, the net energy values of dried grass

Table 21. Armsby's factors for calculating the net energy value of roughage

(kcal. per 100 g.)

Metabolizable energy of digestible organic matter	282-297
Mean value from 106 experiments	288
Heat increment of dry matter ingested	78-125
Mean value	103

declines as the level of feeding is increased. It is difficult, therefore, to compare the net energy values which were determined directly in Exps. (3), (5) and (4) with those computed from the digestibility data by the methods of Kellner and Armsby because of some uncertainty as to the levels of feeding at which the prediction factors are applicable. Further, the present net energy values were measured with grass as the sole constituent of the diet and not as an addition to a basic ration.

As already explained (p. 58) net energy value can be calculated from the exponential equation relating energy retention to feed intake, either as an integral value between any two levels of feeding or as a differential value at any particular level of feeding. The integral value ($NEV_{(1)}$) between planes of nutrition (P) of -0.5 and 0 (p. 60) is given by,

$$NEV_{(1)} = 100 \frac{E_x}{E(D_n - D_x)} \text{ kcal. per 100 g. dry matter.}$$

(The symbols are explained in the footnote overleaf). This method gives values which may best be compared with Armsby's estimates since they refer to a sub-maintenance plane of nutrition. The integral values ($NEV_{(2)}$) between planes of nutrition of 0 and +0.5, which may best be compared with Kellner's

Table 28. Net energy values (kcal./100 g. dry matter) obtained in the present work by direct measurement and also as predicted by Kellner's and Armby's methods

Grass	Predicted values		Direct measurements			
	NEV_K	NEV_A	NEV(1)	NEV(2)	NEV_d	100k(1-B)
B (chopped)	138	133	142	63	115	-105
C (coarse cubes)	152	91	139	116	124	- 85
D (chopped)	152	103	161	116	161	-159
E (coarse cubes)	154	148	165	151	168	-103
F (fine cubes)	154	140	150	150	102	-119
H (chopped)	132	150	171	130	156	- 69

Note: NEV_K is the net energy value predicted by Kellner's method.

NEV_A is the net energy value predicted by Armby's method.

NEV(1) is the net energy value calculated below maintenance.

NEV(2) is the net energy value calculated above maintenance.

NEV_d is the differential net energy value at maintenance (plane of nutrition, P = 0).

When energy retention is n times the net energy requirement for maintenance (P = n), then

NEV_d = (NEV_d at P = 0) + n x 100k(1-B).

estimates are given by,

$$NEV_{(E)} = 100\frac{S_y}{E(D_y - D_m)} \text{ kcal. per 100 g. dry matter.}$$

Net energy values have been calculated in these ways for the grasses used in the present experiments, and in addition, values have been calculated for the same grasses by means of the Kellner and Armsby factors already described. The results are given in the first four columns of Table 22.

It will be seen from the table that the net energy values predicted by the methods of Kellner (NEV_K) and Armsby (NEV_A) differed considerably. For grass O, for example, the two values were 153 and 91 kcal. per 100 g. dry matter, respectively, although

---

Footnote:

S_x and S_y are the energy retentions at D_x and D_y.

S_0 is the energy loss at fasting.

P (plane of nutrition) $= \frac{S_x}{S_0}$

D_m (the dry matter intake at maintenance, $P = 0$)

$$= \frac{1}{k} \cdot \ln\left(\frac{E}{L}\right)$$

D_x (the dry matter intake at $P = -0.5$)

$$= \frac{1}{k} \cdot \ln\left(\frac{E}{L + 0.5S_0}\right)$$

D_y (the dry matter intake at $P = +0.5$)

$$= \frac{1}{k} \cdot \ln\left(\frac{E}{L - 0.5S_0}\right)$$

$S_0 = E - L$

E, L and k are the constants in the exponential equation relating energy retention to dry matter intake.

for grass E the values, 188 and 159, were much closer together. If the two estimates differed only because Kellner's values refer to levels of feeding above maintenance while Armsby's values refer to levels of feeding below maintenance, then the Kellner values should be consistently lower than the Armsby ones, but Table 32 shows that in four of the six comparisons the reverse was true, which indicates inaccuracy in the methods of prediction. This is confirmed when the predicted net energy values are compared with the most appropriate (as explained above) directly-determined values. Thus the net energy values predicted by Armsby's method were 60 to 95% of the corresponding directly-determined values, $NEV_{(1)}$, while the values predicted by Kellner's method were 99 to 316% of the corresponding directly-determined values, NEV(2). The unreliability of the predicted net energy values is not surprising when it is remembered that the methods of prediction were based on very limited information (Tables 30 and 31). However, they have often been adjusted in an arbitrary way in an effort to make them more applicable to present-day needs (see for example ref. 17). In addition, in the estimation of net energy values for practical purposes, digestibilities are obtained from tables, the additive nature of the individual values of the different components of complex rations

is taken for granted and the effects of level of feeding are largely ignored (99). It is unfortunate that it is necessary to use such estimates of food value in the investigation of some aspects of animal nutrition, because comparisons which depend on the use of such inaccurate figures are of limited value.

In view of the effect of level of feeding on net energy, it is necessary to list net energy values for a particular plane of nutrition and to describe their variation with plane of nutrition. One way of doing this is to give the differential net energy value (NEV_d) at maintenance (P = 0) and also the rate of change of this value with plane of nutrition P.

Thus, when the plane of nutrition is zero,

$$NEV_d = 100\,k_L \text{ kcal. per 100 g. dry matter,}$$

and when the plane of nutrition is P,

$$NEV_d = 100k[L + P(L - B)] \text{ kcal. per 100 g.}$$

dry matter (the symbols were described in the footnote to p. 89).

These quantities are given for the several grasses in the last two columns of Table 28. Thus for grass G, NEV_d at P = 0 was 124 kcal. per 100 g. dry matter and at P = 1 (a positive energy retention equal to the net energy requirement for maintenance)

the value was 134 - 1 x 65 = 69 kcal. per 100 g. dry matter. It should be emphasised that until more information is available to describe the effect of one food on the utilisation of the energy of another food to which it is added, this system, or any other, can only be applied to rations whose net energy has actually been measured. That is, it cannot be assumed that the net energy values of the individual components of a ration may be added together to give the net energy value of the ration.

Chapter 2

The energy exchange of fasting sheep

In the measurement of net energy it is important to know whether the basal metabolism of the experimental animals changes during the course of an experiment. For example, if an experiment was made in which a sheep was given first 1000 g. of food per day and then 1500 g., and in which the corresponding energy retentions were zero and 500 kcal. per 24 hr., then the net energy value of the food would be calculated as $100(500 - 0)/(1500 - 1000) = 100$ kcal. per 100 g. of food. However, if the basal energy loss was 1500 kcal. per 24 hr. at the start of the experiment and 1000 kcal. per 24 hr. at the finish, then the real net energy value would be only $100(500 - 500)/(1500 - 1000) = 40$ kcal. per 100 g. of food. Undetected changes in the basal metabolism of the experimental animals could thus lead to serious errors in the estimation of net energy.

In this chapter, the fasting energy losses of the sheep which were determined in the course of Exps. (1)-(4) (listed in Appendix Table 6) will be discussed in relation to (a) the duration of the fasting period, (b) the training of the sheep to accustom them to the respiration apparatus and (c) changes after an interval of several months.

Table 25. The daily heat production on the first 4 days of starvation (mean values for 14 steers)

No. of days of starvation	Mean heat production (kcal. per 24 hr.)
1	1328
2	1140
3	1115
4	1085
Standard error for comparisons of any two means	±20.8

The difference between day 1 and days 2, 3 or 4 is significant at the 0.1% level of probability.

The differences among days 2, 3 and 4 are not significant at the 5% level of probability.

The effects of the duration of the fasting period

The values of the fasting energy exchange which were used in Expts. (1)-(5) were the mean values for the 3rd and 4th days of fasting, the sheep having been fed at the maintenance level for at least 10 days before the period of starvation. That the metabolism was relatively stable on the 3rd and 4th days of fasting is shown by the figures given in Table 35. This table shows that the mean heat productions of 14 sheep on the 2nd, 3rd and 4th days of fasting were 1140, 1115 and 1083 kcal. per 24 hr., respectively. These three values did not differ significantly, but the value for the 1st day was significantly higher at 1238 kcal. per 24 hr.

The details of the changes in oxygen consumption and methane production during the period of fasting with one of the sheep have already been given in Fig. 5 (facing p. 42). These results, which are typical of those obtained with all the sheep, show the rapid decline of the gaseous exchange during the first 3 days of starvation and its relative stability during the second 3 days. In most of the experiments, methane production fell to the very low levels of 1 or 2 litres per 24 hr. and the non-protein respiratory quotient (N.P.Q.) approached a value of 0.7 (an N.P.Q. of 0.707 indicates catabolism of fat only). Similar results to these were obtained by

Blaxter (14) in several short-term experiments with sheep.

While with ruminant animals the attainment of a true post-absorptive state is practically impossible within a reasonable period of fasting, because food residues continue to be fermented in the rumen for many days, this condition was approached quite closely on the 3rd and 4th days of starvation with the sheep used in the present work.

The effect of training

It has already been shown in Part I (p. 41) that training the sheep to accustom them to the respiration apparatus has a very marked effect on their fasting energy expenditure. Thus, as summarised in Table 4, the mean fasting heat production of six untrained sheep was some 30% higher than that of the same sheep when they were trained for a period of 4 weeks. It is noteworthy that the mean fasting heat production of the 19 trained sheep used in Exps. (1)-(4) and also of the two sheep used by Blaxter (14), was 59 kcal. per 24 hr. per kg.$^{0.73}$ of body weight, while the value for the basal metabolism of sheep given by Brody (24) was 72 kcal. per 24 hr. per kg.$^{0.73}$. The latter value seems much too high to be near the true basal metabolism and it may be that it refers to untrained sheep.

It is probable that, even with trained sheep,

Table 24. The fasting heat productions of three trained sheep, measured in two periods 9 months apart

	Sheep no.	Fasting heat production	
		kcal. per 24 hr.	kcal. per 24 hr. per kg.$^{0.75}$
First period	16	1015	65.9
	17	913	63.1
	18	1027	64.1
Second period (9 months later)	16	1122	69.0
	17	1219	72.6
	18	1103	68.0

the minimal resting metabolism (true basal metabolism) is very seldom attained. However, in the estimation of net energy values failure to reach such basal conditions is not of great moment; it is much more important that values should be obtained for the post-absorptive energy exchange under conditions similar to those existing when the energy exchange of the fed animals is measured.

Variations over a period of time

It so happened that the fasting heat production of three of the 19 sheep used in the present work was determined on two occasions 9 months apart. The results for these three sheep, in Table 24, give some indication of the variations which could occur in the course of a long experiment. As shown in this table, the fasting heat production of sheep 17 rose from 913 to 1210 kcal. per 24 hr. in 9 months, while that of sheep 18 rose only from 1087 to 1105. If these increases had been solely due to increases in the body weight of the animals, then the heat productions per kg.$^{0.73}$ of body weight would have remained constant (24). However, as shown in the table, while this was approximately true for sheep 18, the values for the other two sheep increased considerably with time. It may be that the basal metabolism of these sheep was affected by some factor other than gain of body substance, such as, for

example, a temporary (seasonal) or permanent alteration in the endocrine systems of the animals. These results suggest that it is not desirable to rely on the use of energy exchange per kg.$^{0.75}$ of body weight in comparing energy exchanges measured during a period of several months. In addition, body weight is an ill-defined quantity unless the amounts of digesta are known or invariant, neither of which conditions is often fulfilled with ruminant animals. In view of these facts, it is important to keep experiments that are designed to measure net energy as short as possible and thus reduce the complications that arise as a result of changes in the basal metabolism of the experimental animals.

Chapter 5

Errors which arise in the determination of net energy values

In the determination of net energy values a large number of errors contribute to the total error involved. There are instrumental and analytical errors, and errors which are inherent in the methods of computing the energy exchange. In addition, the metabolism of the experimental animals varies from day to day and animals respond differently to the same treatment. It is important to know the magnitude of these errors if the net energy values which are determined are to be properly assessed and if the apparatus, methods and experimental design are to be improved.

The object of this chapter is to examine the errors which arose in the course of the work already described and to discuss them in relation to the measurement of net energy values.

Instrumental errors

Subjective instrumental errors can arise in making and recording the various measurements and other errors can arise because of the physical and chemical limitations of the apparatus used. The subjective errors give rise to random variations which may be greatly reduced and indeed practically eliminated by careful work. The errors due to the

limitations of the apparatus could cause systematic errors in the final results. However, they can be measured and specified, and often eliminated, by the use of suitable tests.

The testing of the respiration apparatus has already been described (p. 24); the results showed that as much as 99% of a quantity of carbon dioxide admitted to the apparatus could be recovered. The volume of the apparatus was measured by three methods (see Table 1 facing p. 27), two of which gave results that differed by about 1%: the third method gave unreliable results for reasons which were explained (p. 27). The volume of the apparatus obtained by the two reliable methods was reduced further by 60 l. to allow for the volume occupied by a sheep. Since the sizes of the sheep differed somewhat, the application of this constant correction may have introduced a further error of about $\pm 0.5\%$ in the volume of the chamber. The calculations that involved the use of the volume of the respiration chamber could thus be in error to the extent of rather more than ± 0.5 due to these causes. These are (a) the calculation of methane production and (b) the calculation of the corrections applied to the apparent carbon dioxide production and oxygen consumption to allow for changes in the composition of the air in the apparatus between the beginning

Table 55. An example of the computation of individual analytical errors of ±0.5% to obtain the resultant errors attached to the energy retention calculated by the R.Q. and O/N methods

		R.Q. estimate of energy retention (kcal. per 24 hr.)	O/N estimate of energy retention	
			Nitrogen exchange (g. per 24 hr.)	Carbon exchange (g. per 24 hr.)
Intake		4295 ± 22	36.4 ± 0.18	423 ± 2.1
Losses:	Faeces	1256 ± 6	6.3 ± 0.04	123 ± 0.6
	Urine	280 ± 1	16.0 ± 0.08	33 ± 0.1
	Methane	836 ± 8		10 ± 0.1
	Heat	2304 ± 10		
	CO_2			227 ± 1.1
Retentions*		429 ± 55	3.3 ± 0.16	34 ± 3.5
				409 ± 54 kcal. per 24 hr.

* The errors of the retentions = $\sqrt{\Sigma E^2}$, where E refers to the individual errors.

and end of a day's experiment. However, 1 or 2% error in these relatively small components of the energy exchange (see below, p.101) would contribute an insignificant amount to the total error attached to the energy retention.

The spirometer that was used to measure the oxygen consumed by the sheep was calibrated by two methods (described on p. 25) which gave results differing by about 1%. The heat production calculated by the R.Q. method could thus be in error to a maximum extent of ±0.5% due to this cause.

Analytical accuracy

In the determination of the carbon and nitrogen contents and the calorific values of food and excreta the maximum analytical error was ±0.5% of the quantity measured. This applies to the checks against the analytical standards and also to replication. In Table 25 an example is given of the summation of analytical errors of this sign to give the resulting errors attached to the estimates of energy retention. This shows that, (at an energy intake of 4296 kcal. per 24 hr.), an error of ±25 kcal. per 24 hr. in the energy retention estimated by the R.Q. method could arise from these small analytical inaccuracies, errors in estimating the energy intake, heat production and faecal energy components being the most important. The corresponding error of the C/N estimate of the energy retention was rather larger,

466 kcal. per 24 hr. (Table 25), and the errors attached to the intakes of carbon and nitrogen, urinary nitrogen, faecal carbon and carbon in respiratory carbon dioxide were the most important components. The maximum analytical errors that could have been attached to the estimates of energy exchange per 24 hr. made in Expts. (3)-(6) were actually much smaller than those given in Table 25 because those estimates were the means of measurements made over periods of several days.

Because each estimate of energy retention depends on so many measurements, analytical accuracy at every stage is exceedingly important. However, since the individual components of the error contribute to the total in proportion to their squares, it is not necessary to obtain as high a degree of accuracy in the measurement of those quantities which make up only a small part of the energy exchange as in the measurement of those quantities which make up a large part of it. Thus in most experiments methane production, and urinary carbon and calorific value were determined with slightly less precision than the other quantities without reducing significantly the accuracy of the estimates of energy retention.

Errors of computation

The theoretical validity of the calculation of

Table 20. The incidence of differences of various magnitudes between the energy retention estimated by the B.S. and C/N methods in the present work

Differences (kcal. per 24 hr.)	No. of times these differences occurred				
	Exp. (1)	Exp. (2)	Exp. (3)	Exp. (4)	Total
Less than 10	0	2	3	1	6
10–50	1	4	14	5	24
50–100	1	3	7	3	14 (31)
100–200	2	5	3	4	14
More than 200	0	0	3	0	3

energy retention by the R.Q. and O/N methods has already been discussed on p. 36, but it is necessary to test the practical validity of the methods. This can be done by comparing the heat production measured by direct calorimetry with that calculated by the R.Q. method or with that obtained by subtracting the energy retention estimated by the O/N method from the metabolizable energy. These comparisons could not be made in the present experiments, but other workers with cattle (4, 34, 35 and 37) have found differences of about 2% of the energy intake, independent of plane of nutrition. In the present work, however, the energy retention estimated by the O/N method was, on the average, 16 kcal. per 24 hr. (0.6% of the average energy intake) higher than that estimated by the R.Q. method, and the magnitude of the discrepancy was not dependent on the plane of nutrition. In the experiments of Lund with pigs kept at high planes of nutrition (39), in which slightly different factors were used to obtain the energy retention by the O/N method, the corresponding figure was 88 kcal. per 24 hr. The incidence of differences of various magnitudes between the two estimates of energy retention, as found in Exps. (1)-(6), are given in Table 30. This table shows that for a total of 61 comparisons, in only three instances did the discrepancy exceed 200 kcal. per

24 hr. (about 5% of the average energy intake of a sheep).

These considerations suggest that there is still a small systematic error in the estimation of energy retention by the C/N method even when factors based on the most up-to-date information (38) are used. Random errors due to this cause and due to errors in the estimation of energy retention by the R.Q. method in the present experiments seldom exceeded 3% of the energy intake, and were more often less than 1%.

Variations between analytical periods

As described on p. 12, each experiment was divided into a number of periods each of which corresponded to a particular treatment and the energy losses on any treatment were measured in experimental periods made up from two or more analytical periods each lasting several days. The variations in the energy losses due to any one treatment from analytical period to analytical period were obtained by statistical analysis of the results of each experiment. The total variance was divided into two parts due to (a) variation between sheep and between treatments and (b) within-sheep variation. In Table 27 the within-sheep errors are given for the faecal energy, heat production (R.Q. estimate), urinary energy, methane energy and energy retention for each experiment, expressed as the standard error of the mean

Table 27. Standard errors (within-sheep) of the mean value of the various components of the energy exchange for any two analytical periods, obtained from the results of Expts. (1)-(4) (cal. per 24 hour)

Experiment	No. of analytical periods per experimental period	Length of the analytical period (days)	Standard error (within-sheep) of the mean of any two analytical periods (± kcal. per 24 hr.)					
			Faecal energy	Heat	Urinary energy	Methane energy	Total*	Energy retention
1(b)	6	3	76	83	16	11	85	108
2(sheep B)	8	3	58	86	10	7	80	68
2(sheep S)	8	3	35	82	6	6	88	86
3	5	3	122	—	16	—	127	145
3	2	3	—	80	—	16		
4	1	6	67	—	11	—	122	75
4	1	4	—	75	—	16		

* $\sqrt{(F^2 + H^2 + U^2 + M^2)}$ where F, H, U and M are the errors of the faecal energy, heat production, urinary energy and methane energy, respectively.

The standard error for the mean of the actual number (n) of analytical periods in any particular experiment, E = S.E. $\sqrt{8/n}$ where S.E. is a standard error from the table.

of any two analytical periods. The numbers of analytical periods per experimental period are also given so that the actual standard errors attached to the mean values of the energy exchange (R.Q. estimate) can be calculated for each experiment. For example, the standard error of a mean energy retention in Exp. (1(b) was $\pm$ 103 x $\sqrt{3/6}$ = $\pm$ 89 kcal. per 24 hr. because there were six analytical periods per experimental period.

Faecal errors ranged from $\pm$ 26 to $\pm$ 183 kcal. per 24 hr. In Exp. (4) (p. 79) a reasonable degree of accuracy was obtained (standard error $\pm$ 67 kcal. per 24 hr.) although there was only one analytical period per experimental period: the same is true of the errors attached to the urinary energy losses, which ranged from $\pm$ 6 to $\pm$ 18 kcal. per 24 hr. Heat production was subject to much less variation than faecal losses (except in Exp. (4)), the errors lying between $\pm$ 23 and $\pm$ 75 kcal. per 24 hr. The errors attached to the energy lost as methane ($\pm$ 7 to $\pm$ 16 kcal. per 24 hr.) were in most cases a little smaller than those involved in the measurement of urinary energy. Finally, the errors attached to the energy retention varied from $\pm$ 56 to $\pm$ 146 kcal. per 24 hr. The summation of the errors of the individual components of the energy loss[x] ($\pm$ 85 to $\pm$ 127 kcal. per

[x] $\sqrt{F^2 + H^2 + U^2 + M^2}$ where F, H, U and M are the errors of the faecal energy, heat production, urinary energy and methane energy respectively.

24 hr.) agreed reasonably well with the actual errors of the energy retention, which indicates that the error involved in estimating the mean energy intake per 24 hr. was not large.

The effects of the length of the analytical period on the accuracy of the various measurements

The only quantities for which a sufficient number of serial 24 hr. measurements were available to permit statistical analysis were urinary nitrogen, heat production (R.Q. estimate) and carbon dioxide production. For each of these components the variance due to day-to-day variations (irrespective of any changes with level of feeding) was obtained in the same way as was the variance due to variations between analytical periods discussed above. The analysis was made with the 24 hr. values and also with the 48 hr. values obtained by grouping them into pairs. The standard deviations of the 24 hr. values and of the 48 hr. values were then compared in order to determine the effect of doubling the period of measurement.

With heat productions calculated by the R.Q. method from the urinary nitrogen and respiratory exchange (161 degrees of freedom for the error term obtained from the 24 hr. values), the standard deviation of a 24 hr. value was ± 52 kcal. per 24 hr. and the standard deviation of a 48 hr. value was

± 56 kcal. per 24 hr. Thus, since $52/\sqrt{2} \sim 36$, the standard error of a mean of n 24 hr. measurements was approximately ± $52/\sqrt{n}$ kcal. per 24 hr.

A similar result was obtained for carbon dioxide production (150 degrees of freedom for the error term obtained from the 24 hr. values), the standard error of a mean of n 24 hr. measurements being ± $36/\sqrt{n}$ litres per 24 hr. which is equivalent to ± $360/\sqrt{n}$ kcal. per 24 hr. in terms of energy retention as body fat. This error is much larger than any of the others encountered, a fact that is due partly to analytical difficulties. Thus, in order to remove all the water vapour produced by the sheep and to avoid its retention in the carbon dioxide absorbers, the calcium chloride in the absorption bottles used in these experiments (Fig. 3) must be very porous and the individual pieces must be less than 3 cm. in diameter. Again, a very sensitive but robust balance is needed to weigh the five 11 kg. bottles comprising the carbon dioxide absorbers to obtain an accurate measure of perhaps 600 g. of carbon dioxide. However, it appears from this result that carbon dioxide production would have to be measured for very long periods (n = 50 or 60 days) to make the standard error of a mean 24 hr. value in terms of the energy retention as fat comparable to the error of the heat production

Table 28. Standard errors* of the mean values of the various components of the energy exchange, for any two sheep given the same treatment, obtained from results with 16 sheep

Component of energy exchange	Standard error of mean for any two sheep (± kcal. per 24 hr.)
Faecal energy	41
Heat production	42
Urinary energy	16
Methane energy	19
Energy retention	60

* Calculated from the analysis of variance as

$$[(c) - (d)] / \sqrt{2n}$$

where (c) is the between-sheep variance, (d) is an error variance term (see text p.108) and n is the number of analytical periods per experiment with each sheep.

calculated by the M.Q. method.

This statistical analysis could not be made with faecal energy loss because sufficient data were not available. However, relevant information was obtained in another experiment (20) which showed that, irrespective of level of feeding, the standard error of the mean of n 24 hr. measurements with sheep given dried grass D was $\pm$ 28/n g. of faecal dry matter per 24 hr. rather than $\pm$ 28/$\sqrt{n}$. This was because the error resulted from the chance of one defecation too few or too many being collected at the beginning and end of each analytical period. Since the calorific value of the faecal dry matter was relatively constant from day to day in Exp. (1b) at 4.40 $\pm$ 0.08 kcal. per g., the standard error of the mean of n 24 hr. measurements of the faecal energy loss may be taken as about $\pm$ 100/n kcal. per 24 hr.

With urinary nitrogen 69 degrees of freedom were available for the error obtained from the 24 hr. values, and it seemed again that the standard error of the mean of n 24 hr. measurements was $\pm$ 0.65/n g. nitrogen per 24 hr., rather than $\pm$ 0.65/$\sqrt{n}$. This was equivalent to an error of $\pm$ 22/n kcal. per 24 hr. in terms of energy retention as body protein.

Variation between sheep

In Expts. (5) and (4) and in several pre-starvation periods a number of sheep were given identical rations and their energy exchange measured. The

results from these experiments were analysed statistically to obtain the variations between sheep receiving the same treatment. The total variance was divided into four parts, (a) between experiments, (b) between analytical periods, (c) between sheep and (d) an error term. The estimated variance from which the error due to variations between sheep was calculated consisted of,

$$\left[(c) - (d)\right] / \sqrt{2n},$$

where n was the number of analytical periods per experiment with each sheep.

The standard errors of the means for any two sheep (calculated as described above from the results obtained from 16 sheep) for the components of the energy exchange are given in Table 28. These errors were similar to the within-sheep errors already described (see Table 27). For faecal energy loss and energy retention (N.G. estimate), the between-sheep errors ($\pm$ 41 and $\pm$ 50 kcal. per 24 hr. respectively) tended to be smaller than the corresponding within-sheep errors ($\pm$ 85 to $\pm$ 183 and $\pm$ 86 to $\pm$ 145 kcal. per 24 hr.). For the heat production (N.G. estimate) the reverse was true, the between-sheep error being $\pm$ 44 kcal. per 24 hr. and the within-sheep errors being $\pm$ 88 to $\pm$ 75 kcal. per 24 hr. The between-sheep errors

attached to the urinary energy (± 16 kcal. per 24 hr.) and methane production (± 10 kcal. per 24 hr.) were a good deal smaller than the other errors and similar to the corresponding within-sheep errors.

The planning of experiments to measure net energy

In planning experiments to measure the net energy value of food given to sheep, several factors must be considered if reliable results are to be obtained. First, in order to establish metabolic equilibrium, each experimental ration must be given to the sheep for at least 10 days before any measurements of energy retention are made. Secondly, in order to maintain the calorimetric apparatus in good working order it is necessary to allow 1 day per week for cleaning it and for repairing any faults which develope: if Saturday is set aside for this purpose weekend work is minimised, a fact which is important in prolonged experiments. Thirdly, at least with sheep given dried grass, the within-sheep error attached to an estimate of the mean daily energy retention, which is approximately equal to the sum of the errors attached to the estimates of faecal energy loss and heat production, decreased only slowly as the period of measurement is increased beyond 6 days. Thus, on the basis of the errors which arose in the present work (see p.105ff), this error would be about ± 60 kcal. per 24 hr. for measurements made over 3

days and ± 40, ± 27, ± 20 and ± 17 kcal. per 24 hr. for measurements made over 3, 6, 9 and 12 days, respectively.

Taking account of these three points, the choice of experimental systems for the measurement of energy retention may, in the first instance, be limited to (a) systems involving measurement of the energy exchange of each sheep on each ration for 3 consecutive days, with four or more sheep for replication, and (b) systems involving measurements for 6 days with three or more sheep for replication. The within-sheep errors would be about ⅓ smaller with the longer periods of measurement and if the length of the period on each ration was sufficient, could be reduced further with little additional effort, by collecting the faeces for a longer period than allowed for the measurement of heat production and only analysing one mixture of faeces representing the whole period of collection.

If it is assumed that the within- and between-sheep errors given on p. 104 and p. 108 apply equally to all levels of nutrition, the standard error which would be attached to the mean net energy value of a dried grass measured by any one of these systems can be calculated approximately. For example (see calculation on p. A3 of the Appendix), if the energy retentions of four sheep were measured by the

R.Q. method over 5-day periods at each of two levels of feeding a dried grass (say 1000 and 1500 g. per day), then the standard error attached to the mean net energy value of the grass (say 150 kcal. per 100 g.) would be about ± 11 kcal. per 100 g. or about ± 7%. However, if measurements of the fasting energy losses of the sheep were also made and the net energy value calculated from an equation relating energy retention to food intake, then, apart from any effects on net energy due to level of feeding, the standard error would be reduced to about ± 8% of the net energy value. Although it is not possible to say whether net energy measurements made with foods other than dried grass would have the same levels of error, these approximate errors are very much smaller than the error of ± 20% which may well be attached to net energy values computed from digestibility data by the commonly used method of Kellner (15, 53).

Conclusions

(1) The foregoing analysis of the errors which arose in the present work, showed that, when instrumental and analytical errors were small, the error attached to a mean energy retention was mainly determined by first, the number of sheep used and second, the length of the periods of measurement of

faecal energy loss and heat production. In the present work the average standard error of a mean energy retention estimated by the R.Q. method, due to variations between two sheep given the same ration, was ± 80 kcal. per 24 hr., and the standard error (within-sheep) of the mean energy retention of a sheep measured over 3 days was also about ± 60 kcal. per 24 hr.

(2) Comparisons of the energy retentions estimated by the R.Q. and C/N methods gave no indication of large systematic error in the computations. The C/N method gave energy retentions which were on the average only 16 kcal. per 24 hr. higher than those obtained by the R.Q. method. However, the rather large error which arose in the measurement of carbon dioxide production (standard deviation ± 30 litres per 24 hr.) caused the energy retention estimated by the C/N method to be somewhat less accurate than that estimated by the R.Q. method.

(3) The error of an average net energy value for dried grass was shown to be about ± 3% when measurements made at three levels of feeding and with four sheep were used in its calculation: when only two levels of feeding were used the error was about ± 7%.

Chapter 4

General conclusions

The purpose of the work described in the foregoing chapters was the development of a method for food evaluation on a net energy basis, which would give reliable results and which could be used readily on a large scale for the study of many feedingstuffs. The main results of the experiments and the more important points of the discussion of these results will now be brought together to show to what extent this purpose has been achieved.

Conditions necessary for the measurement of net energy

Before a measurement of energy retention can be made the experimental animal must be in a stable metabolic condition. The losses of energy from the body can be related to a particular feeding regimen only when the effects due to the previous regimen have been eliminated. In ruminants the time required for this is governed largely by the rate of passage of the food residues from the rumen. It has been shown elsewhere (20) that sheep require about 10 days to eliminate the residues of a meal from the digestive system and results obtained in the present experiments confirmed that in sheep this is the length of time needed for the establishment of metabolic equilibrium following any considerable

change of diet.

It is most important also that each animal used for the tests should have a constant level of basal energy expenditure throughout an experiment. If the basal energy loss alters by an unknown amount during the periods that must elapse between the measurements of the energy retention promoted by the food, then the net energy value which is obtained for the food will be incorrect. Thus, for example, only animals which have been carefully trained to accustom them to the experimental routine should be used, for it was found that, with animals which are untrained at the start of an experiment, considerable variations occur in basal energy loss.

The experiments showed that, at normal levels of feeding, the net energy value of chopped dried grass was not altered by grinding and cubing it nor was it affected by alterations in the frequency of feeding. At very high levels of feeding, cubed dried grass was utilized slightly more efficiently than the same dried grass fed in the chopped form, and it may be that with foods of a different quality from those used here, the cubing process would have a larger effect on their net energy values. However, if it is generally true that the physical condition of a food does not affect its net energy value to any significant extent, then there are several reasons for using cubed rations for

experimental purposes. For example, cubed rations can be prepared, sampled and stored more easily than rations composed of chopped fodder: there is also no possibility of selective refusal of part of the food by the animal. Further, higher levels of feeding are often attained with cubed material because of its more rapid passage through the digestive tract (20) and perhaps because it may sometimes be more palatable than chopped food.

A study of the rate of methane production during the 24 hr. of the day showed that there were considerable changes from hour to hour, related to the frequency of feeding. It has been shown elsewhere (20) that the pattern of food ingestion influences the passage of food through the digestive tract and therefore the digestion of the food and the metabolism of the animal. To avoid unnecessary sources of variation it is thus desirable that feeding, collection of excreta and measurement of respiratory exchange should follow a fixed daily routine and that measurement of the energy exchange should be extended over at least 24 hr.

The effect of level of feeding on net energy

It was shown that as the level of feeding was increased the net energy value of the food decreased to a considerable extent, and an exponential equation was used to describe the curvilinear relationship between energy retention and food intake. This

equation was developed to meet various needs. First, by means of the equation a simple description was provided of net energy value at the maintenance level of feeding and of the way in which this value changed with plane of nutrition. Secondly, comparisons between animals of different sizes were made possible by modifying the terms of the exponential equation in accordance with the basal energy expenditure of the animals. Thirdly, the equation allowed comparisons to be made, at the appropriate planes of nutrition, between the net energy values which were determined directly in the present work and those which were predicted from digestibility data by the commonly-used methods of Kellner (55) and Armsby (5).

When the exponential function is used to relate energy retention to food intake, energy retention must be measured at not less than three levels of feeding - preferably at four widely-separated levels if a high degree of accuracy is to be achieved. This means that, with only a small amount of replication among different animals (say four sheep), and with the shortest possible preliminary and experimental periods (10 and 5 days, respectively), an experiment lasting 8 to 10 weeks would be required to produce the net energy value of one food if one respiration chamber were used. A net energy value measured in this way at three levels

of feeding would have a standard error of about ± 5%: net energy values predicted from digestibility data by the methods of Kellner and Armsby have been said to be in error to the extent of ± 20% (18) and certainly, with the grasses used in the present work, the predicted values differed widely from those determined directly.

The usefulness and reliability of the methods used to measure net energy in the present experiments

On the basis of the work described in this thesis it may be said that closed-circuit respiration calorimetry is eminently suitable for the measurement of net energy values with small farm animals and on a large scale. The respiration chambers proved reasonably easy to operate over long periods and permitted the determination of the heat production of the experimental animals with a degree of accuracy that was comparable to that obtained by means of direct calorimetry (errors of 1 or 2%).

Because of the large number of measurements involved in each estimation of energy retention, a high degree of accuracy is necessary at every stage and, in the present experiments, most analyses were accurate to within ± 0.5% of the quantities. Although with this degree of accuracy in the methods of analysis, good agreement (about 3 or 5% of the energy intake) was found between the energy retention estimated by the R.Q. method and that

obtained by the C/N balance technique, the latter was subject to a larger error, mainly because of the large day-to-day variations in the estimate of the carbon lost in the excreted carbon dioxide.

Since the R.Q. method of estimating the energy retention was the more accurate and since it was found to be simpler to use than the C/N method, the R.Q. method might well be employed exclusively when net energy is being measured on a wide scale, with a considerable saving of time and labour. If the carbon retention were not measured the energy storage in body fat could be calculated as the difference between the total energy retention and the energy storage in body protein.

Kellner and Armsby made great advances in their day, but it has to be recognised that large errors exist in predicting net energy values by their methods, and it is most important that direct measurements of the utilisation of the energy of food should be continued. The present work has shown that suitable methods are now available for work with small farm animals such as sheep, and indicates that in such experimentation it is of the greatest importance to have a complete description of the relationship between net energy and plane of nutrition if the results are to have any real validity and usefulness.

Summary of the thesis

1. The various technical terms used throughout this work are defined on p. i .

2. The object of the work described in the present thesis was the development of a method for the measurement of net energy, suitable for work on an extensive scale with small farm animals. The need for this work is discussed in the introduction.

3. To this end the utilisation of the energy of dried grass by fattening sheep was studied. The closed-circuit respiration chambers used to measure the respiratory exchange and hence the energy retention of the sheep, and the analytical methods are described in Part I. The methods of calculating the energy retention from the intake and losses of energy (E.G. method) and from the storage of protein and fat (C/N method) are also discussed critically.

4. The various experiments made are described in Part II of the thesis.
Experiment (1 a) showed that the animals must be carefully trained to accustom them to the experimental routine if large changes in their basal metabolism during experiments are to be avoided. It was concluded from Exp. (1 b) that the sheep should receive the particular ration

being studied for at least 10 days before measurements of the energy exchange are made. In Experiments (2), (3) and (4) the effects of level of feeding, of the physical form of the food and of the frequency of feeding were studied. Experiment (2) showed that net energy decreased as food intake increased, mainly due to the digestibility of the food decreasing and the heat increment becoming greater as the level of intake was raised. These effects were confirmed in Experiments (3) and (4). The relationship between energy retention and energy intake was described by an exponential equation.

Cubed grass was found in Experiments (2) and (3) to be less digestible and to give a lower heat increment than chopped grass. The main factor responsible for these effects was the rate of passage of the food through the digestive tract. The net energy value of the grass was not affected by its physical form at normal levels of feeding but at very high levels, chopped grass had a slightly lower value than the same grass when cubed.

In Experiment (4) it was shown that frequency of feeding had no significant effect on energy utilisation except that methane production was slightly depressed when one large meal was given each day instead of four small ones.

5. In Part III of the thesis the results of the experiments are discussed further in relation to the prediction and direct measurement of net energy values.

The net energy values which were determined directly are compared with those calculated from digestibility data by the well-known methods of Kellner and Armsby. Serious disagreement between these values is shown to exist and the causes of it are discussed. A system of expressing net energy values based on the use of the exponential equation relating energy retention to food intake is described. This system takes account of the variation of net energy with food intake.

The usefulness of the measurements of the fasting energy expenditure of the sheep that were made in the several experiments is also discussed. It is shown that the energy expenditure on the 3rd and 4th days of fasting approximated to the true basal metabolism of the sheep and was a relatively constant quantity which could be used as the energy expenditure at the zero level of feeding in the estimation of net energy.

Analytical errors were reduced to $\pm$ 0.5% of the quantities measured and the main errors in the experiments arose from variations from day to

day and from sheep to sheep in the loss of energy in the faeces and in the heat production. The energy retention estimated by the O/N method was less accurate than that estimated by the R.Q. method, mainly because of the difficulty of measuring the respiratory loss of carbon as carbon dioxide. There was no large systematic error in the methods of estimating energy retention.

6. It is concluded that the energy retention of sheep can be measured accurately and relatively easily using the methods described. To achieve an accuracy of about ± 3% in the estimation of net energy and to take account of the effect of level of feeding, energy retention should be measured at not less than three levels of feeding and the experiments should be replicated among four sheep that have been specially trained to accustom them to the routine involved in the work.

References

1. Anderson, A.C. (1933). Biochem. J. 167, 145.

2. A.O.A.C. (1947). Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, 5th ed. Washington D.C.

3. Armsby, H.P. (1904). Mem. Penn. St. Coll. agric. Exp. Sta. 13, 1057.

4. Armsby, H.P. (1913). J. Amer. chem. Soc. 35, 1794.

5. Armsby, H.P. (1917). The Nutrition of Farm Animals. New York: The Macmillan Co.

6. Axelsson, J. (1945). K. Lantbrukst. Annl. Stockh. 24, 259.

7. Axelsson, J. (1949). Lantbrukst. Ann. 16, 84.

8. Axelsson, J. (1949). Lantbrukst. Ann. 16, 404.

9. Axelsson, J. & Eriksson, S. (1953). Lantbrukst. Ann. 20, 61.

10. Benedict, F.G. (1938). Nutr. Bull. nat. Acad. Sci. No. 19.

11. Benedict, F.G. & Collins, W.E., Hendry, M.F. & Johnson, A. (1939). Tech. Bull. N.H. agric. Exp. Sta. No. 16.

12. Benedict, F.G. & Mitchell, H.C. (1926). Proc. nat. Acad. Sci., Wash. 12, 604.

13. Benninger, F.H. & Kästinger, C. (1949). Rev. Sci. Instr. 20, 649.

14. Blaxter, K.L. (1948). J. agric. Sci. 38, 207.

15. Blaxter, K.L. (1950-51). Nutr. Abstr. Rev. 20, 1.

16. Blaxter, K.L. & Graham, N.McC. (1955). J. agric. Sci. 46, 293.

17. Blaxter, K.L. & Graham, N.McC. (1956). Proc. Nutr. Soc. 14, 181.

18. Blaxter, K.L. & Graham, N.McC. (1956). J. agric. Sci. 47, 207.

19. Blaxter, K.L., Graham, N.McC. & Rook, J.A.F. (1954). J. agric. Sci. 45, 10.

20. Blaxter, K.L., Graham, N.McC. & Wainman, F.W. (1956). Brit. J. Nutr. 10, 69.

21. Blaxter, K.L. & Hovell, A.B. (1951). Brit. J. Nutr. 5, 25.

22. Blaxter, K.L. & Rook, J.A.F. (1953). Brit. J. Nutr. 7, 83.

23. Bloomer, T.M., Murdoch, F.R., Lintott, R.E., Nyo, K.S. & Shaw, A.O. (1953). J. Dairy Sci. 36, 515.

24. Brody, S. (1945). Bioenergetics and Growth. New York: Reinhold.

25. Capstick, J.W. (1951). J. agric. Sci. 41, 400.

26. Carpenter, T.M., Lee, R.C. & Pinnoxby, A.E. (1950). Tierernähr. u. Fütter. (Abt. B) 4, 1.

27. Carrol, E.J. & Rupgate, R.E. (1954). Applied Microbiol. 2, 205.

28. Cate, H.A., Lewis, J.M., Webb, B.J., Wakefield, M.E. & Garrigus, V.S. (1955). J. Anim. Sci. 14, 187.

29. Cipolloni, E.M., Schneider, B.H., Lucas, H.L. & Pavlech, H.E. (1951). J. Anim. Sci. 10, 667.

30. Crasemann, E. (1955). Landw. Forsch. No. 5.

31. Ellis, G.H., Matrone, G. & Maynard, L.A. (1946). J. Anim. Sci. 5, 295.

32. Forbes, E.B. (1955). Science. 77, 300.

33. Forbes, E.B., Braman, W.W. & French, R.B. (1927). J. agric. Res. 34, 333.

34. Forbes, E.B., Braman, W.W. & Kriss, M. (1938). J. agric. Res. 57, 253.

35. Forbes, E.B., Braman, W.W. & Kriss, M. (1939). J. agric. Res. 40, 67.

36. Forbes, E.B., Braman, W.W., Kriss, M. & Swift, R.W. (1943). J. agric. Res. 66, 753.

37. Forbes, E.B., Fries, J.A. & Braman, W.W. (1938). J. agric. Res. 31, 987.

58. Forbes, R.B., Fries, J.A. & Braman, W.W. & Kriss, R.V. (1936). J. agric. Res. 52, 685.

59. Forbes, R.B. & Kriss, M. (1938). J. Nutr. 9, 185.

60. Forbes, R.B., Kriss, M. & Miller, R.C. (1944). J. Nutr. 9, 535.

61. Forbes, R.B. & Swift, R.V. (1941). Bull. Pa. Agric. Exp. Sta. No. 415.

62. Fraps, G.S. (1937). Proc. Amer. Soc. Anim. Prod. 30, 20.

63. Fries, J.A. (1932). Proc. Amer. Soc. Anim. Prod. 55.

64. Gjon, A. (1911). Pflüg. Arch. ges Physiol. 140, 309.

65. Gordon, J.G. & Tribe, D.E. (1953). Aust. J. Nutr. 6, 89.

66. Grouven, H. (1864). Ber. Landbauen. Versuchs-Stationen 2, 207.

67. Hagemann, O. (1911). Landw. Jb. 41, Erg. bd. 1, 1.

68. Hallsworth, E.G. (1949). J. agric. Sci. 39, 884.

69. Hansson, E. (1916). Handbok i Utfodringslära. Stockholm: G.E. Fritzes Bokförlags Aktiebolag.

70. Hellberg, A. (1949). Metabolism of Rabbits at Different Planes of Nutrition. Uppsala: Almqvist & Wiksells Boktryckeri.

71. Henneberg, W., Kuhn, G., Merker, ., Schulze, & Schulze, (1859). J. Landw. 17, 176.

72. Hoshiko, Y.T., Hashizume, S. & others. (1955). Bull. nat. Inst. agr. Sci. Japan. Ser. G.11, 157.

73. Kellner, O. (1920). Die Ernährung der landwirtschaftlichen Nutztiere. 9th ed. Berlin: Paul Parey.

74. Kirchgessner, M. (1956). Arch. Tierernähr. 6, 160.

75. Kleiber, M. (1929). Forschg. Landw. 4, 55.

76. Kleiber, M. (1955). Hilgardia, 9, 1.

57. Kleiber, M. (1945-6). Hubr. Abstr. Rev. 16, 207.

58. Kleiber, M., Regan, W.M. & Mead, S.W. (1948). Hilgardia 18, 511.

59. Krebs, H.A. (1954). Johns Hopk. Hosp. Bull. 95, 19.

60. Kriss, M. (1931). J. Nutr. 4, 141.

61. Kuhn, O. (1894). Landw. Versuchsw. 44, 257.

62. Kullgren, O. & Tyden, H. (1939). IngenVetensk-Akad. Handl. No. 94.

63. Lasnaigne, (1846). J. Chim. med. 22, 477 & 751; 23, 15 & 553.

64. Lasnaigne, (1849). J. Pract. chem. 46, 257; 47, 156.

65. Lassiter, C.A., Denton, T.W. & Ruck, J.W. (1955). J. Dairy Sci. 38, 1249.

66. Lefevre, J. & Auguet, A. (1939). Arm. Physiol. Physiochim. biol. 5, 513.

67. Lines, E.W. & Pierce, A.W. (1931). Bull. Comm. sci. industr. Res. Aust. No. 55.

68. Loewy, A. (1911). Oppenheimers Handb. Biochem. 4, 133.

69. Lund, A. (1938). Bergen. Forsongslab. Hdn. No. 180.

70. Marston, H.R. (1948). Aust. J. sci. Res., Ser. B. 1, 93.

71. Maynard, L.A. (1953). J. Nutr. 51, 15.

72. Meigs, E.B. (1925). J. Dairy Sci. 8, 533.

73. Mitchell, H.H. (1934). Science, 80, 556.

74. Mitchell, H.H. (1948). J. Anim. Sci. 1, 159.

75. Mitchell, H.H., Hamilton, T.S. & Haines, W.T. (1932). J. agric. Res. 45, 165.

76. Mitchell, H.H., Hamilton, T.S. & Haines, W.T. (1941). J. Nutr. 23, 541.

77. Mollgaard, H. (1939). Fütterungslehre des Milchviehs. Hannover: M. & H. Schaper.

78. Mollgaard, H. & Andersen, A.C. (1947). K. Veterinär. Aarsskr. 195.

79. Nehring, K. & others (1954). Festschrift anlässlich des 100 jährigen Bestehens der Landwirtschaftlichen Versuchsstation Leipzig-Möckern, Vols. 1 & 2. Berlin: Deutscher Bauernverlag.

80. Norman, A.G. & Jenkins, S.H. (1938). Biochem. J. 32, 818.

81. Pilgrim, A.F. (1948). Aust. J. sci. Res., Ser. B. 1. 150.

82. Pregl, F. (1924). Quantitative Organic Microanalysis. London: J. & A. Churchill.

83. Regnault, V. & Reiset, J. (1849). Ann. Chim. (Phys) 26, 299.

84. Rubner, M. (1894). Z. Biol. 30. 73.

85. Rubner, M. (1903). Die Gesetze des Energieverbrauches bei der Ernährung. Leipzig u. Wien: F. Deuticke.

86. Schatermikoff, M.N., Molleschanowa & Tomme, (1927). J. Biol. Med. exp., Moscou 7, 375.

87. Schneider, B.H. (1947). Feeds of the World. Morgantown: W. Va. agric. Exp. Sta.

88. Soskine, S. & Levine, R. (1946). Carbohydrate Metabolism. Chicago: University of Chicago Press.

89. Soxhlet, (1878). Ber. landw.-chem. Vers Stn., Wien 1, 101.

90. Stohmann, F. (1876). Landw. Versuchsw. 19, 81.

91. Swift, R.W. (1937). Bull. Penn. St. Coll. agric. Exp. Sta. No. 615.

92. Tangl, F. (1912). Biochem. Z. 44, 365.

93. Thorn, J.A. & Shu, P. (1951). Quand. J. Chem. 29, 556.

94. Voit, C. (1891). Physiologie des Gesamtstoffwechsels.

95. Velbo, R. & Boyd, J. (1955). J. Sci. Fed. Army. 4, 197.

96. Werner, A. & Franke, K.R. (1954). Denkschrift anlässlich des 100 jährigen Bestehens der Landwirtschaftlichen Versuchsstation Leipzig-Möckern. Vol. 1. Berlin: Deutscher Bauern-verlag.

97. Wagner, G. & Ghumeln, A. (1950). Tierernährung 2, 195.

98. Woodman, H.E. (1942). Bull. Minist. Agric., Lond. No. 122.

99. Woodman, H.E. (1948). Bull. Minist. Agric., Lond. no. 48, 11th ed.

100. Zuntz, N. (1918). Landw. Jb. 44, 778.

101. Zuntz, N. & Schumburg, H. (1901). Physiologie des Marsches. Berlin: Hirschwald.

Appendix

The method used to fit the exponential equation, $S = I - Be^{-kI}$, to the relationship between energy retention, S, and energy intake, I, in Exp. 2

First, an approximation to the value of k was obtained:

$$dS/dI = kBe^{-kI}$$

when $I = 0$ $\quad dS/dI = kB$

and when $S = 0$ $\quad dS/dI = k$

$$\therefore (dS/dI)_{I=0} = (dS/dI)_{S=0} = k(S - I_{S=0}) = -kI_{S=0}$$

Substitution of actual values (see below) gave,

$$k \sim -\left(\frac{976 - 355}{1951 - 0} - \frac{186 - 92}{3843 - 2891}\right)/976 \sim -3 \times 10^{-4}$$

The data for sheep 6 were:

I (kcal. per 24 hr.)	S	kI	e^{-kI}	B_x (see A2)	$S_{est.}$	$S - S_{est.}$
0	-976	0	1.000	2659	-1064	+108
1951	-355	0.5903	0.677	1707	-232	-123
2835	+92	0.8650	0.568	1500	+55	+36
2891	+88	0.8758	0.561	1481	+74	-46
3843	+186	0.7664	0.464	1234	+331	-195
4738	+504	0.9446	0.369	1026	+389	+95
5604	+746	1.1608	0.313	827	+726	+18
6701	+693	1.3692	0.267	679	+976	+16
7695	+100	1.5210	0.218	577	+970	+123

(a) A linear regression, S = L + Mx, was fitted to the data.

$$M = \frac{\Sigma(x - \bar{x})(s - \bar{s})}{\Sigma(x - \bar{x})^2} = 3669, \text{ and}$$

$$L = \bar{s} + M\bar{x} = 1656.$$

(b) Values of s were estimated from the regression as $s_{est.} = 1656 - 3669x$.

(c) The algebraic sum (Q) of $\Sigma x(s - s_{est.})$ was -874,677; since this was negative the value of k that was used was too large.

(d) The steps (a), (b) and (c) were repeated with other values of k until Q approximated to zero. When this was achieved the equation was

$$s = 6436 - 4404s^{-0.621 \times 10^{-4}}$$

Calculation of the error that would be attached to two estimates of the net energy value of a dried grass

If the estimated within-sheep variance is σ^2_e, and the estimated between-sheep variance is σ^2_n, the total error variance is then $\sigma^2_e + \sigma^2_n \frac{\sqrt{sn-1}}{sn-1}$, where s is the number of sheep used and n is the number of days for measurement of the energy exchange on each occasion.

The standard deviation of an estimate of faecal energy loss, due to within-sheep variations was found to be $\pm$ 100 kcal. per 24 hr. (p. 107); the figure for heat production was $\pm$ 58 kcal. per 24 hr. (p. 105). The standard deviations due to variations between sheep were $\pm$ 41 kcal. per 24 hr. for faecal energy and $\pm$ 44 kcal. per 24 hr. for heat production (Table 39).

Thus the estimated variance of faecal energy loss determined for 5 days with each of four sheep would be

$$100^2 + \frac{5\sqrt{6}}{11} \cdot 41^2 = 12353$$

and for heat production the variance would be,

$$58^2 + \frac{5\sqrt{6}}{11} \cdot 44^2 = 5298.$$

Therefore the standard error of the mean energy retention would be

$$\sqrt{\frac{12252 + 5292}{4 \times 5}} = \pm 36 \text{ kcal. per 24 hr.}$$

Now if energy retention were measured at two food intakes of 1000 and 1500 g. per 24 hr., then the standard error of the mean net energy value would be

$$\pm 36\sqrt{2}/500 = \pm 11 \text{ kcal. per 100 g.}$$

However, if three levels of nutrition were used instead of two, e.g. 0, 1000 and 1500 g. per 24 hr., then, apart from any effects on net energy due to level of feeding, the standard error of a net energy value calculated from an equation relating energy retention to food intake would be approximately

$$\pm 36\sqrt{3}.\sqrt{2/3}/1500 = \pm 6 \text{ kcal. per 100 g.}$$

Appendix Table 1. Chemical composition of the dried grasses (as % of dry matter)

Constituent	Grass A (chopped)	Grass B (chopped)	Grass C (coarse cubes)	Grass D (chopped)	Grass D (coarse cubes)	Grass D (fine cubes)	Grass E (chopped)
Proximate analysis:							
Organic matter	89.6	89.2	89.4	92.7	91.8	92.5	89.6
Ether extract	4.9	5.0	5.6	4.1	4.7	4.4	2.8
Crude protein	16.8	16.9	17.9	13.6	14.2	15.0	17.5
Crude fibre	27.8	26.6	26.0	22.1	21.3	21.1	28.9
Nitrogen-free extract	40.5	40.6	42.0	52.7	51.6	58.0	45.6
Detailed analysis:							
Hexose sugar	1.7	2.5	2.1		5.3		1.6
Sucrose	2.7	7.6	6.0		4.7		0.0
Fructosan	0.3	0.9	0.6		5.0		0.0
Cellulose	87.3	84.8	80.8		28.7		28.6
Cellulosic furfural	5.6	8.1	7.4		4.7		4.6
Non-cellulosic furfural	7.4	5.5	4.8		5.4		4.5
Lignin	5.0	5.7	7.7		5.4		4.5
Carbon	45.0	44.9	44.2		45.6		44.8
Calorific value (kcal.)	445.0	440.0	441.0		441.0		450.0

Appendix Table 2. Digestibility data

Exp. no.	Grass	Sheep no.	Food Intake (g D.E. per 24 hr)	% Digestibility								
				D.M.	Organic matter	Ether extr.	Crude fibre	Crude protein	N free extr.	Cellulose	Cell. furfural	Non-cell. furfural
1(b)	A (before fasting)	8	1,054	62.1	63.4	65.9	59.2	71.9	63.0	62.8	3.4	74.1
	(after fasting)		1,054	57.9	58.9	66.3	58.7	65.0	59.2	59.9	15.0	72.3
2	B	2	884	72.7	75.6	77.1	61.4	72.1	72.3	62.3	64.2	79.6
	C	3	871	58.3	61.9	75.4	55.7	66.9	55.9	65.6	39.6	75.4
3	D (chopped)	14	1,268	76.9	77.9	52.4	77.3	61.4	84.5	62.6	76.6	77.4
		15	1,240	73.7	75.5	45.7	73.6	60.1	83.0	74.9	73.9	73.9
		16	1,279	77.3	79.3	75.7	79.5	61.6	84.3	86.6	80.6	79.5
		17	562	65.0	84.9	73.5	85.2	66.6	90.1	89.6	90.0	85.0
		18	562	79.5	81.4	62.1	80.1	65.0	80.6	85.9	68.5	84.6
		19	562	79.4	81.9	62.6	82.5	69.2	86.4	87.2	89.6	76.9
	(coarse cubes)	14	1,372	69.1	71.2	71.7	60.9	60.0	77.2	59.6	69.5	66.4
		15	1,380	67.7	69.2	65.1	56.3	59.3	76.0	65.7	62.9	67.5
		16	1,372	66.3	68.5	75.1	58.9	56.8	74.8	65.5	72.3	69.4
		17	554	74.3	77.3	61.2	75.6	67.3	82.2	82.2	83.1	76.1
		18	554	71.5	75.3	72.0	72.9	57.1	81.8	78.4	76.7	75.5
		19	554	75.4	76.8	51.2	75.9	61.4	81.4	81.1	76.2	76.1
	(fine cubes)	14	1,377	66.8	68.7	66.9	62.9	66.9	77.8	62.3	51.9	69.6
		15	1,377	64.9	65.2	56.3	50.9	55.1	74.2	54.0	48.7	56.6
		16	1,377	62.2	63.6	60.9	46.1	52.3	73.5	52.4	48.9	54.1
		17	546	75.6	77.5	66.7	71.8	66.6	85.0	77.8	75.2	77.5
		18	543	70.1	72.5	66.8	68.6	60.1	80.8	74.1	71.4	75.8
		19	546	75.5	75.6	70.5	68.8	61.0	82.5	77.1	78.4	75.5
4	E	16	785	74.4	77.1	53.0	81.6	70.7	77.3	85.6	89.3	55.7
		17	785	75.8	78.1	56.7	81.7	72.6	78.5	85.6	88.7	48.9
		18	788	78.7	78.5	50.3	81.8	70.5	78.4	85.1	87.3	50.0
	(one meal per day)	16	949	73.5	76.4	57.0	80.7	71.7	77.4	85.1	87.3	65.1
		17	936	73.5	76.5	66.0	81.5	68.1	77.7	87.9	89.0	69.3
		18	951	72.5	75.6	50.9	78.2	70.1	77.8	85.2	87.4	59.6
	(two meals per day)	16	951	77.4	79.7	63.2	83.7	74.9	80.4	87.5	90.2	64.5
		17	950	75.0	77.6	61.1	82.3	71.5	78.4	85.4	87.3	67.6
		18	951	75.5	78.7	62.9	82.3	71.9	80.5	85.4	85.8	58.4
	(four meals per day)	16	956	73.0	75.9	80.9	80.5	64.6	79.4	84.5	85.4	62.8
		17	951	72.3	75.6	58.5	80.3	69.7	76.6	83.0	83.5	64.5
		18	954	73.1	75.9	76.1	77.4	68.8	77.6	84.4	86.0	62.9

Appendix Table 2.

Exp. no.	Grass	Sheep no.	Energy intake	Energy loss				Energy retention		Non-protein R.Q.	Body weight (kg.)
				Faeces	Urine	Methane	Heat prod.	R.Q. est.	C/N est.		
1(b)	A (before fasting)	3	4689	1955	205	251	1778	+506	+554	1.01	47.9
	(after fasting)		4389	2074	225	219	1705	+457	+260	1.05	48.7
2	B	2	2834	507	159	162	1305	- 79	- 85	0.86	39.0
			2787	768	196	219	1596	- 12	+ 84	0.95	46.2
			3716	1107	330	270	1565	+244	+395	0.97	44.4
			4461	1575	284	332	2127	+343	+454	1.02	48.4
			5430	1627	514	376	2463	+589	+620	1.05	48.1
	C	3	1951	787	131	135	1250	-353	-356	0.84	37.1
			2625	1086	142	174	1538	+ 91	+287	0.96	48.5
			2591	1096	154	180	1592	+ 66	- 11	0.96	39.2
			3842	1642	209	214	1641	+136	- 6	1.00	37.3
			4723	1955	227	255	1845	+492	+515	1.02	48.1
			5604	2396	256	281	2035	+746	+685	1.06	41.6
			6791	2741	359	356	2435	+904	+680	1.06	46.6
			7605	3287	412	347	2559	+1100	+999	1.11	49.7
3	D (chopped)	14	5556	1496	341	465	2807	+587	+722	1.04	51.4
		15	5494	1552	278	386	2874	+684	+625	1.05	53.3
		16	5538	1422	248	439	2875	+959	+1169	1.01	54.7
		17	2389	567	135	206	1482	0	- 11	1.02	48.0
		18	2389	575	115	202	1599	+100	+ 43	1.01	44.8
		19	2389	528	115	185	1426	+136	+179	0.99	44.7
	(coarse cubes)	14	6044	2020	319	415	2865	+925	+1584	1.01	54.4
		15	6044	2083	285	261	3156	+819	+826	1.12	53.5
		16	6044	3115	254	369	2852	+1014	+1035	1.10	50.2
		17	2441	665	120	204	1661	+ 88	+118	0.99	48.9
		18	2441	775	115	174	1392	- 6	+ 81	0.94	45.1
		19	2441	757	65	152	1341	+148	+128	0.98	45.7
	(fine cubes)	14	6218	2180	331	389	2497	+831	+867	1.07	53.7
		15	6218	2557	319	215	2524	+975	+575	1.24	52.0
		16	6218	3562	240	257	2208	+951	+940	1.06	50.1
		17	2462	663	126	200	1582	+ 91	+115	0.99	49.0
		18	2462	696	115	207	1548	+ 96	+104	0.97	44.7
		19	2462	715	106	172	1629	+140	+150	0.97	45.6

Continued on next page/

Appendix Table 3.

Appendix Table 5 (contd.)

Exp. no.	Grass	Sheep no.	Energy intake	Energy loss				Energy retention		Non-protein R.Q.	Body weight (kg.)
				Faeces	Urine	Methane	Heat prod.	R.Q. est.	C/N est.		
4	E	16	3434	976	285	266	1897	+133	+ 75	1.03	52.7
		17	3486	929	324	262	1860	+151	+353	0.96	47.1
		18	3475	921	245	262	1678	+116	+148	1.01	47.1
	(one meal per day)	16	4279	1105	248	295	2866	+575	+484	1.04	57.2
		17	4215	1021	256	309	2285	+304	+518	0.96	51.4
		18	4289	1245	259	299	2032	+455	+554	1.04	51.7
	(two meals per day)	16	4287	1251	275	344	2134	+259	+152	1.01	55.6
		17	4279	1145	275	316	2304	+341	+361	0.98	55.6
		18	4292	1249	252	340	1952	+476	+491	1.06	54.6
	(four meals per day)	16	4292	1244	290	335	2092	+331	+284	1.04	60.2
		17	4286	1126	296	344	2104	+411	+449	1.00	58.5
		18	4295	1256	269	326	2004	+429	+409	1.02	56.4

Note: Heat production was calculated from oxygen consumption as shown in Table 5.
R.Q. estimate of energy retention is energy intake less energy losses.
C/N estimate of energy retention is the energy stored in body fat and protein.
Non-protein R.Q. was calculated from the oxygen consumption and carbon dioxide production adjusted to complete oxidation of methane.

Appendix Table 5 (contd.).

Exp. no.	Grass	Sheep no.	Nitrogen intake	Nitrogen loss		Nitrogen retention
				Faeces	Urine	
1(b)	A (before fasting)	3	28.73	9.14	15.00	+6.59
	(after fasting)		28.73	9.86	15.99	+4.89
2	B	2	11.87	2.91	7.44	+1.78
			17.00	4.65	12.34	+0.01
			21.95	6.28	11.47	+4.21
			26.55	7.88	17.77	+1.21
			31.85	9.30	18.05	+4.76
	C	3	18.60	3.95	9.20	-0.55
			18.17	5.72	13.06	-0.61
			19.17	5.53	13.24	+0.40
			24.60	6.43	14.82	+1.35
			30.67	9.36	17.75	+3.57
			38.67	11.65	20.24	+7.52
			41.67	13.96	23.41	+5.87
			46.64	16.04	29.72	+8.73
3	D (chopped)	14	28.05	10.79	19.53	+1.34
		15	27.74	10.93	12.96	+3.85
		16	28.47	10.88	12.03	+5.56
		17	12.07	4.03	7.15	+0.89
		18	12.07	4.19	6.83	+1.05
		19	12.07	3.69	7.63	+0.75
	(coarse cubes)	14	30.99	11.56	14.11	+5.32
		15	28.92	12.45	12.27	+4.17
		16	30.99	13.49	14.71	+2.79
		17	12.52	4.09	6.98	+1.45
		18	12.52	5.57	6.40	+0.75
	(fine cubes)	14	30.44	13.08	14.30	+3.06
		15	30.44	13.64	12.76	+4.04
		16	30.44	14.19	15.62	+2.63
		17	12.05	4.03	6.95	+1.07
		18	12.05	4.75	6.51	+0.79
		19	12.05	4.71	6.96	-0.58

Continued on next page/

Appendix Table 4.

Appendix Table 4 (contd.)

Exp. no.	Grass	Sheep no.	Nitrogen intake	Nitrogen loss		Nitrogen retention
				Faeces	Urine	
4	E	16	31.57	6.88	12.06	+5.19
		17	21.58	5.92	11.45	+4.31
		18	31.51	6.41	15.73	+1.87
	(one meal per day)	16	26.28	7.61	14.60	+4.07
		17	25.58	6.80	17.59	+1.23
		18	26.28	9.55	17.08	-0.85
	(two meals per day)	16	26.57	8.41	16.96	+1.09
		17	26.88	7.31	16.20	+3.57
		18	26.59	7.98	16.19	+3.28
	(four meals per day)	16	26.39	7.88	16.75	+1.76
		17	26.34	7.38	17.89	+1.07
		18	26.40	8.24	15.97	+2.19

Appendix Table 4 (contd.).

Exp. no.	Grass	Sheep no.	Carbon intake	Carbon loss				Carbon retention
				Faeces	Urine	Methane	Carbon dioxide	
1(b)	A (before fasting)	3	478.8	198.0	20.4	14.4	196.0	+48.0
	(after fasting)		478.8	200.4	25.8	12.5	194.6	+25.0
2	B	2	195.7	48.9	14.0	9.5	130.8	- 5.8
			268.7	78.0	22.5	12.5	170.9	+ 2.8
			377.1	106.8	22.5	15.4	198.9	+33.8
			458.1	135.8	29.8	19.0	240.7	+33.8
			544.4	160.1	31.0	21.5	279.8	+52.0
	C	3	193.8	79.3	13.3	7.6	121.5	-28.5
			257.0	110.0	16.9	10.2	149.1	- 1.5
			289.8	114.8	15.7	10.0	130.2	+15.3
			384.5	170.1	22.0	12.5	179.9	+ 0.2
			486.9	199.9	23.1	14.6	206.5	+48.9
			581.8	245.9	29.6	16.0	236.2	+54.1
			684.5	279.3	53.6	20.4	278.0	+75.5
			787.5	329.5	40.9	19.5	292.9	+84.4
3	D (chopped)	14	587.7	144.4	34.7	26.8	312.8	+68.2
		15	567.9	155.1	29.8	20.9	312.8	+51.9
		16	583.6	141.6	26.5	25.1	294.2	+96.2
		17	247.3	54.4	14.4	11.6	167.5	- 0.4
		18	247.3	55.7	14.5	11.5	151.3	+ 4.0
		19	247.3	51.6	14.8	10.6	155.9	+14.7
	(coarse cubes)	14	620.6	170.5	38.9	25.7	262.2	+124.3
		15	575.9	206.2	30.7	14.9	256.4	+69.1
		16	620.6	216.4	29.7	21.1	267.9	+85.6
		17	250.6	64.4	14.1	11.7	150.7	+ 9.7
		18	250.6	74.4	14.2	9.9	145.3	+ 6.9
		19	250.6	68.9	14.6	9.2	146.7	+11.3
	(fine cubes)	14	683.8	212.4	35.0	22.3	296.4	+70.7
		15	638.8	244.8	33.7	14.9	292.3	+48.2
		16	658.8	265.4	27.0	14.7	260.3	+76.4
		17	251.0	82.7	15.9	11.5	153.2	+ 9.7
		18	251.0	69.1	12.8	11.8	147.9	+ 9.4
		19	251.0	70.2	15.1	9.8	143.8	+12.1

Continued on next page/

Appendix Table 5.

Appendix Table 5 (contd.)

Exp. no.	Grass	Sheep no.	Carbon intake	Carbon loss				Carbon retention
				Faeces	Urine	Methane	Carbon dioxide	
4	E	16	561.5	94.8	24.0	15.2	219.6	+ 7.7
		17	561.5	89.8	23.1	15.0	208.4	+31.2
		18	550.6	95.9	23.1	15.0	210.8	+12.5
	(one meal per day)	16	426.8	107.3	25.6	16.3	256.0	+40.6
		17	419.6	99.7	27.9	17.5	248.7	+25.7
		18	427.0	116.8	14.9	17.6	231.6	+44.1
	(two meals per day)	16	426.6	120.8	27.7	19.6	246.0	+12.7
		17	426.6	110.0	25.9	18.1	241.8	+30.2
		18	427.6	122.0	24.8	19.4	228.4	+35.2
	(four meals per day)	16	427.2	120.1	27.7	19.2	240.6	+19.6
		17	426.4	107.1	27.7	19.6	235.7	+36.3
		18	427.6	122.0	25.8	18.6	227.4	+53.8

Appendix Table 5 (contd.).

(kcal. per 24 hr)

Exp. no.	Sheep no.	Body weight (kg)	Energy loss					R.Q.
			Methane	Urine	Heat prod.	Heat prod. / 0.75 kg.	Total	
1(b)	3	45.6	15	63	895	57.3	976	0.71
2	2	57.1	12	52	921	65.8	985	0.67
3	14 U*	57.3	18	95	1,366	71.1	1,478	0.66
	14	62.7	24	66	1,015	69.5	1,105	0.72
	15 U	48.8	15	86	1,395	81.5	1,494	0.69
	15	58.6	18	11	844	46.1	873	0.69
	16 U	48.4	15	86	1,209	71.1	1,308	0.67
	16	55.4	18	44	1,015	55.9	1,077	0.65
	17 U	48.3	17	68	1,194	70.6	1,349	0.75
	17	49.2	24	76	913	55.1	1,018	0.64
	18 U	44.8	15	52	1,112	69.9	1,179	0.70
	18	44.8	11	61	1,027	64.1	1,099	0.69
	19 U	44.7	18	76	1,067	67.9	1,158	0.78
	19	42.8	12	53	889	57.4	954	0.72
4	16 U	49.6	15	98	1,315	76.0	1,428	0.77
	16	56.2	18	96	1,122	60.0	1,236	0.78
	17 U	44.2	19	69	1,313	82.5	1,401	0.75
	17	47.5	15	105	1,210	73.9	1,350	0.76
	18 U	42.8	17	86	1,189	75.7	1,302	0.69
	18	51.2	21	153	1,103	62.2	1,286	0.73
	19 U	42.4	19	80	1,066	69.8	1,165	0.75

* U indicates animals not recently accustomed to experimental conditions

Appendix Table 6.

Appendix Table 7. Rate of methane production over 24 hours and frequency of feeding

No. of meals per day	Methane production per 2 hours as % of the 24 hour total				Time after 1st meal (hr)
	Sheep 16	Sheep 17	Sheep 18	Mean	
1 (at 0 hr)	9.97	8.31	11.24	9.84	0
	8.56	7.44	8.82	8.47	2
	9.04	9.96	11.02	10.01	4
	8.95	10.40	7.95	9.10	6
	9.52	12.52	10.76	10.67	8
	10.24	7.25	7.77	8.42	10
	7.40	7.96	9.35	8.28	12
	9.44	8.76	7.20	8.46	14
	5.76	8.52	6.41	6.90	16
	5.22	5.72	5.22	5.39	18
	7.67	6.33	5.69	5.90	20
	7.93	6.96	8.74	7.88	22
2 (at 0 h and 12 hr)	9.26	5.54	6.65	7.25	0
	7.17	12.52	7.73	9.07	2
	6.69	7.91	6.77	7.12	4
	11.73	7.15	7.94	8.96	6
	6.55	10.03	7.06	7.81	8
	5.26	7.51	6.73	6.45	10
	10.52	9.99	8.48	9.66	12
	6.82	6.79	8.57	7.59	14
	7.17	6.31	6.14	6.54	16
	8.39	10.29	5.43	8.04	18
	9.59	6.70	15.13	10.41	20
	11.26	11.53	11.37	11.38	22

Appendix Table 7.